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1.	Your reference	ANTT / P22728GB
		(4APR00 E526524-1 D02866 P00 0.00-0008049.9
2.	Patent application number *(The Patent Office will fill in this part)*	**0008049.9**
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	Antisoma Research Limited West Africa House Hanger Lane Ealing London W5 3QR United Kingdom
	Patents ADP number *(if you know it)*	7869100001
	If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom
4.	Title of the invention	COMPOUNDS FOR TARGETING
5.	Name of your agent *(if you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	**ERIC POTTER CLARKSON** **PARK VIEW HOUSE** **58 THE ROPEWALK** **NOTTINGHAM** **NG1 5DD**
	Patents ADP number *(if you know it)*	**1305010**

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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*	YES

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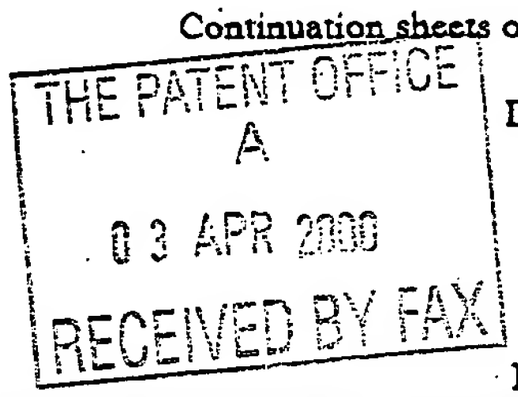

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Abstract	1
Drawing(s)	84

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Priority Documents	0
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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	NO
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11. I/We request the grant of a patent on the basis of this application.

Signature Eric Potter Clarkson Date 3 April 2000

ERIC POTTER CLARKSON

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COMPOUNDS FOR TARGETING

The present invention relates to cytotoxic compounds that have a high avidity for, and can be targeted to, selected cells. Specifically, the invention provides compounds comprising a cytotoxic portion having DNA endonucleolytic activity and a target-cell specific portion having specificity for human polymorphic epithelial mucin (PEM).

Background

The cell-specific targeting of compounds that are directly, or indirectly, cytotoxic has been proposed as a way to combat diseases such as cancer. Bagshawe and his co-workers have disclosed (Bagshawe (1987) *Br. J. Cancer* **56**, 531; Bagshawe *et al* (1988) *Br. J. Cancer* **58**, 700; WO 88/07378) conjugated compounds comprising an antibody or part thereof and an enzyme, the antibody being specific to tumour cell antigens and the enzyme acting to convert an innocuous pro-drug into a cytotoxic compound. The cytotoxic compounds were alkylating agents, *e.g.*. a benzoic acid mustard released from *para*-N-bis(2-chloroethyl)aminobenzoyl glutamic acid by the action of *Pseudomonas sp.* CPG2 enzyme.

An alternative system using different pro-drugs has been disclosed (WO 91/11201) by Epenetos and co-workers. The cytotoxic compounds were cyanogenic monosaccharides or disaccharides, such as the plant compound amygdalin, which release cyanide upon the action of a β-glucosidase and hydroxynitrile lyase.

In a further alternative system, the use of antibody-enzyme conjugates containing the enzyme alkaline phosphatase in conjunction with the pro-drug etoposide 4'-phosphate or 7-(2'-aminoethyl phosphate)mitomycin or a combination thereof have been disclosed (EP 0 302 473; Senter *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4842).

Rybak and co-workers have disclosed (Rybak *et al* (1991) *J. Biol. Chem.* **266**, 21202; WO 91/16069) the cytotoxic potential of a monomeric pancreatic ribonuclease when injected directly into *Xenopus* oocytes and the cytotoxic potential of monomeric RNase coupled to human transferrin or antibodies directed against the transferrin receptor. The monomeric RNase hybrid proteins were cytotoxic to human erythroleukaemia cells *in vitro*.

Other approaches are the *in vivo* application of streptavidin conjugated antibodies followed, after an appropriate period, by radioactive biotin (Hnatowich *et al* (1988) *J. Nucl. Med.* **29**, 1428-1434), or injection of a biotinylated mAb followed by radioactive streptavidin (Paganelli *et al* (1990) *Int. J. Cancer* **45**, 1184-1189). A pilot radioimmunolocalisation study in non-small cell lung carcinomas was conducted with encouraging results (Kalofonos *et al* (1990) *J. Nucl. Med.* **31**, 1791-1796).

Apart from these examples, it is rather more common to see biotinylated antibodies and streptavidin-enzyme conjugates, which are used in enzyme-linked immunosorbent assays.

These previous systems have used relatively large antibody-enzyme,

antibody-streptavidin or antibody-biotin conjugates and may comprise portions of non-mammalian origin which are highly immunoreactive.

We have now devised improved compounds for targeting cells to be destroyed.

Summary of Invention

A first aspect of the invention provides a compound comprising a target cell-specific portion and a cytotoxic portion characterised in that the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof, and the cytotoxic portion has endonucleolytic activity.

By "target cell specific" portion we mean the portion of the compound which comprises one or more binding sites which recognise and bind to polymorphic epithelial mucin (PEM) on the target cell. Upon contact with the target cell, the target cell specific portion is preferably internalised along with the cytotoxic portion. Such internalisation results in the cytotoxic portion being delivered to the cell cytosol, where it has access to the cell's nucleic acid molecules.

The target cell-specific portion of the compounds of the invention comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof.

Polymorphic epithelial mucin, or PEM, is a component of the human milk

fat globule. PEM is expressed by cells in several body tissues and is also found in urine. Significantly, PEM is known to be expressed in epithelial cancer cells, notably in ovarian, gastric, colorectal and pancreatic cancer cells.

Monoclonal antibodies which will bind to PEM are already known, but in any case, with today's techniques in relation to monoclonal antibody technology, antibodies can be prepared to most antigens. The antigen-specific portion may be a whole antibody, a part of an antibody (for example a Fab or $F(ab')_2$ fragment), a synthetic antibody fragment (for example a single chain Fv fragment [ScFv]), or a peptide/peptidomimetic or similar. Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in *"Monoclonal Antibodies: A manual of techniques"*, H Zola (CRC Press, 1988) and in *"Monoclonal Hybridoma Antibodies: Techniques and Applications"*, J G R Hurrell (CRC Press, 1982) and *Antibody Engineering, A Practical Approach*, McCafferty, J. et al, ed. (IRL Pres, 1996).

By 'humanised monoclonal antibody' we include monoclonal antibodies having at least one chain wherein the framework regions are predominantly derived from a first, acceptor monoclonal antibody of human origin and at least one complimentarity-determining region (CDR) is derived from a second, donor monoclonal antibody having specificity for PEM. The donor monoclonal antibody may be of human or non-human origin, for example it may be a murine monoclonal antibody.

Preferably, both chains of the humanised monoclonal antibody comprise

CDRs grafted from a donor monoclonal antibody having specificity for PEM.

Advantageously, the CDR-grafted (*i.e.* humanised) chain comprises two or all three CDRs derived from a donor antibody having specificity for PEM.

Conveniently, the humanised monoclonal antibody comprises only human framework residues and CDRs from a donor antibody having specificity for PEM.

However, it will be appreciated by those skilled in the art that in order to maintain and optimise the specificity of the humanised antibody it may be necessary to alter one or more residues in the framework regions such that they correspond to equivalent residues in the donor antibody.

Conveniently, the framework regions of the humanised antibody are derived from an human IgG monoclonal antibody.

Methods of making humanised monoclonal antibodies are well-known in the art, for example see Jones *et al.* (1986) *Nature* **321**:522-525, Riechmann *et al.* (1988) *Nature* **332**:323-327, Verhoeyen *et al.* (1988) *Science* **239**:1534-1536 and EP 239 400 (to Winter).

In a preferred embodiment of the first aspect of the invention, the target cell-specific portion comprises an humanised HMFG-1 monoclonal antibody or an antigen binding fragment thereof.

HMFG antibodies are raised against human milk fat globule (HMFG), in a delipidated state (see Taylor-Papadimiriou *et al.*, 1981, *Int. J. Cancer* **28**:17-21 and Gendler *et al.*, 1988, *J. Biol. Chem.* **236**:1282-12823). HMFG-1 monoclonal antibodies bind to a particular component of HMFG, namely polymorphic epithelial mucin (PEM). Binding is thought to involve the amino acid sequence APDTR within the twenty amino acid tandem repeats of the *muc-1* gene product.

Examplary humanised HMFG-1 antibodies are disclosed in WO 92/04380.

Advantageously, the target cell-specific portion is an humanised HMFG-1 monoclonal antibody.

In a preferred embodiment of the first aspect of the invention, the target cell-specific portion comprises a fragment of an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), said fragment retaining the antigen binding properties of the parent antibody.

The variable heavy (V_H) and variable light (V_L) domains of the antibody are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by "humanisation" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent parented antibody (Morrison *et al* (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6851-6855).

That antigenic specificity is conferred by variable domains and is independent of the constant domains is known from experiments involving

the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better *et al* (1988) *Science* **240**, 1041); Fv molecules (Skerra *et al* (1988) *Science* **240**, 1038); disulphide-linked Fv molecules (Young *et al.*, 1995, *FEBS Lett.* **377**:135-139); single-chain Fv (ScFv) molecules where the V_H and V_L partner domains are linked via a flexible oligopeptide (Bird *et al* (1988) *Science* **242**, 423; Huston *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward *et al* (1989) *Nature* **341**, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) *Nature* **349**, 293-299.

By "ScFv molecules" we mean molecules wherein the V_H and V_L partner domains are linked via a flexible oligopeptide.

Chimaeric antibodies are discussed by Neuberger *et al* (1988, *8th International Biotechnology Symposium* Part 2, 792-799).

The advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments allows for rapid clearance, and may lead to improved tumour to non-tumour ratios. Fab, Fv, ScFv, disulphide Fv and dAb antibody fragments can all be expressed in and secreted from bacteria, such as *E. coli*, or eukaryotic expression systems such as Yeast or mammalian systems, thus allowing the facile production of large amounts of the said fragments.

Whole antibodies, and $F(ab')_2$ fragments are "bivalent". By "bivalent" we

mean that the said antibodies and $F(ab')_2$ fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv, disulphide Fv and dAb fragments are monovalent, having only one antigen combining site.

Preferably, the target cell-specific portion of the compounds of the invention comprises an antigen binding fragment of the humanised antibody selected from the group consisting of Fab-like molecules, such as Fab and $F(ab')_2$, Fv molecules, disulphide-linked Fv molecules, ScFv molecules and single domain antibodies (dAbs).

More preferably, the target cell-specific portion comprises a Fab molecule or a $F(ab')_2$ molecule.

Yet more preferably, the target cell-specific portion comprises at least a part of one or both of the amino acid sequences shown in Figure 3.

Most preferably, the target cell-specific portion comprises both of the amino acid sequences shown in Figure 3.

Preferably, the target cell-specific portion recognises the target cell with high avidity.

By "high avidity" we mean that the target cell-specific portion recognises the target cell with a binding constant of at least $K_d = 10^{-6}$ M, preferably at least $K_d = 10^{-9}$ M, suitably $K_d = 10^{-10}$ M, more suitably $K_d = 10^{-11}$ M, yet more suitably still $K_d = 10^{-12}$ M, and more preferably $K_d = 10^{-15}$M or even $K_d = 10^{-18}$ M.

Preferably, the target cell-specific portion comprises an antigen binding fragment of an humanised HMFG-1 monoclonal antibody, *e.g.* an Fab or $F(ab')_2$ fragment thereof, wherein a hinge region contains a mutation (*i.e.* wherein the hinge is a variant or hybrid of a naturally occurring hinge). More preferably, the variant hinge comprises the amino acid sequence CCVECPPCPAPE.

By 'cytotoxic portion' we mean a portion having endonucleolytic activity which is toxic to the cell if it is to reach, and preferably enter said cell.

In a preferred embodiment of the first aspect of the invention, the cytotoxic portion has DNA endonucleolytic activity.

Advantageously, the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease.

Examples of known DNA endonucleases include bovine DNase I (see Worrall and Conolly, 1990, *J. Biol. Chem.* **265**:21889-21895). Human pancreatic DNase I has also been cloned (see Shak *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* **87**:9188-9192 and Hubbard *et al.*, 1992, *New Eng. J. Med.* **326**:812-815).

Preferably, the endonuclease is a mammalian deoxyribonuclease I.

More preferably, the endonuclease is a human deoxyribonuclease I.

Most preferably, the cytotoxic portion comprises the amino acid sequence shown in Figure 2.

Preferably, the cytotoxic portion of the compound of the invention is capable of oligomerisation, *e.g.* dimerisation. Attachment of the target-cell specific portion to a cytotoxic portion capable of oligomerisation provides a method for increasing the number of binding sites to the target cell. For example, if the target cell-specific portion is joined to a portion capable of forming a dimer then the number of target cell-specific binding sites is two; if the target cell-specific portion is joined to a portion capable of forming a tetramer then the number of target cell-specific binding sites is four. The number of target cell-specific binding sites is greater than one and the compounds may therefore have a greater avidity for the target cell than do compounds which only have one target cell-specific binding site.

It is preferable for the cytotoxic portion of the compound of the invention capable of oligomerisation to contain no interchain disulphide bonds nor intrachain disulphide bonds; to be well characterised; to be non-toxic; to be stable; to be amenable to preparation in a form suitable for pre-clinical or clinical use or be in pre-clinical or clinical use; and for the subunit monomers to have a high affinity for each other, that is they contain one or more subunit binding sites.

Advantageously, the cytotoxic portion is of mammalian, preferably human, origin. The use of the said mammalian proteins as the cytotoxic portion of the compound of the invention is advantageous since such compounds are less likely to give rise to undesirable immune reactions.

It will be appreciated by those skilled in the art that the cytotoxic portion

may be a variant of a naturally occurring endonuclease.

By "a variant" we include cytotoxic portions comprising of a naturally occurring endonuclease wherein there have been amino acid insertions, deletions or substitutions, either conservative or non-conservative, such that the changes do not substantially reduce the endonuclease activity of the variant compared to that of the naturally occurring endonuclease. For example, the variant may have increased activity compared to the naturally occurring endonuclease

Such variants may be made using methods of protein engineering and site-directed mutagenesis commonly known in the art (for example, see Sambrook *et al.*, 1989, *Molecular cloning: A Laboratory Manual*. 2nd edition, Cold Spring Harbor Laboratory Press, NY, USA).

In an alternative embodiment, the endonuclease is a restriction endonuclease, such as a microbial type II restriction endonuclease. Exemplary type II restriction endonucleases include *Bam*HI, *Hind*III, *Msp*I, *Sau*3AI, *Hinf*I, *Not*I and *Eco*RI.

In another preferred embodiment of the first aspect of the invention, a nuclear localization signal is incorporated into the compound.

Preferably, the nuclear localization signal comprises a nuclear localization signal from the SV40 large T antigen (Kalderon *et al.*, 1984, *Cell* 39:499-509), and specifically the amino acid sequence PKKKRKV. Inclusion of a nuclear localization signal encourages the compound of the invention to gain access to the chromosomal DNA during the periods of the cell sycle

when the nuclear membrane is intact, since the nuclear pores are permeable to large molecules incorporating said nuclear localization signal.

In a further preferred embodiment of the first aspect of the invention, the target cell-specific portion and the cytotoxic portion are fused to create a fusion compound.

By "fusion compound" we include a compound comprising one or more functionally distinct portions, wherein the distinct portions are contained within a single polypeptide chain produced by recombinant DNA techniques.

Preferably, the target-cell specific and the cytotoxic portion of the fusion compound of the invention separated by a linker sequence, for example to allow greater flexibility of the portions relative to one another.

More preferably, the linker sequence comprises a GG dipeptide.

Most preferably the linker sequence is or comprises GG or GSGG.

Alternatively, the target-cell specific and the cytotoxic portion of the compound of the invention are separate moieties linked together by any of the conventional ways of cross-linking polypeptides, such as those generally described in O'Sullivan *et al Anal. Biochem.* (1979) **100**, 100-108. For example, the antibody portion may be enriched with thiol groups and the enzyme portion reacted with a bifunctional agent capable of reacting with those thiol groups, for example the N-hydroxysuccinimide

ester of iodoacetic acid (NHIA) or N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP). Amide and thioether bonds, for example achieved with m-maleimidobenzoyl-N-hydroxysuccinimide ester, are generally more stable *in vivo* than disulphide bonds.

A second aspect of the invention provides a nucleic acid encoding a compound according to the first aspect of the invention, or a target cell-specific portion or cytotoxic portion thereof.

By "nucleic acid molecule" we include DNA, cDNA and mRNA molecules.

A further aspect of the present invention provides a method of making a compound according to the first aspect of the invention, said method comprising expressing one or more nucleic acid molecules according to the second aspect of the invention in a host cell and isolating the compound therefrom.

It is preferable that the two portions of the compound of the invention are produced as a fusion compound by recombinant DNA techniques, whereby a length of DNA comprises respective regions encoding the two portions of the compound of the invention either adjacent one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the compound. The benefits in making the compound of the invention using recombinant DNA techniques are several fold. Firstly, it enables a high degree of precision with which the two portions of the compound can be joined together. Secondly, the construction of compounds which are "hetero-oligomeric" can be

controlled by the expression of the different recombinant DNA molecules encoding each of the different type of subunit of the "hetero-oligomer" in the same host cell.

By "hetero-oligomer" we mean those compounds in which two or more different cell-specific portions are joined to either the same or to different subunits which are capable of oligomerisation. The expression, in the same host cell of two compounds, of A and B, each with different target cell specific portions but with a common second portion capable of oligomerisation will result in a mixed population of compounds. For example, if the common second portion is capable of dimerisation, three potential compounds will be produced: A_2, AB and B_2, in a ratio of 1:2:1, respectively.

The separation of the desired compound with each of the different cell specific portions, that is AB, can be achieved by two step affinity chromatography.

Application of the mixture of compounds to an affinity column specific for A will result in the binding of A_2 and AB. These compounds are eluted from this first column, and then applied to an affinity column specific for B. This will result in AB, but not A_2, being bound to the column. Finally, the desired product AB, can be eluted.

Of course, the order in which the affinity columns are used is not important.

The same principle of separating those compounds with two or more

different binding sites can be applied to the purification of the desired compounds from mixtures of other hetero-oligomers.

Conceivably, the two portions of the compound may overlap wholly or partly.

The nucleic acid is then expressed in a suitable host to produce a polypeptide comprising the compound of the invention. Thus, the nucleic acid encoding the compound of the invention or a portion thereof may be used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the polypeptide of the invention. Such techniques include those disclosed in US Patent Nos. 4,440,859 issued 3 April 1984 to Rutter *et al*, 4,530,901 issued 23 July 1985 to Weissman, 4,582,800 issued 15 April 1986 to Crowl, 4,677,063 issued 30 June 1987 to Mark *et al*, 4,678,751 issued 7 July 1987 to Goeddel, 4,704,362 issued 3 November 1987 to Itakura *et al*, 4,710,463 issued 1 December 1987 to Murray, 4,757,006 issued 12 July 1988 to Toole, Jr. *et al*, 4,766,075 issued 23 August 1988 to Goeddel *et al* and 4,810,648 issued 7 March 1989 to Stalker, all of which are incorporated herein by reference.

The nucleic acid encoding the compound of the invention or a portion thereof may be joined to a wide variety of other nucleic acid sequences for introduction into an appropriate host. The companion nucleic acid will depend upon the nature of the host, the manner of the introduction of the nucleic acid into the host, and whether episomal maintenance or integration is desired.

It will be appreciated that in order to prevent expression of the cytotoxic portion of the compound of the invention from killing the host cells in which it is expressed, it may be necessary to link the nucleic acid of the second aspect of the invention to a signal sequence capable of directing secretion of the expressed compound (or portion) out of the host cell. Signal sequences will be selected according to the type of host cell used. Exemplary signal sequences include the *ompA* signal sequence (for example, see Takahara *et al*..1985. *J. Biol. Chem.* **260(5)**:2670-2674).

Generally, the nucleic acid is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the nucleic acid may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognised by the desired host, although such controls are generally available in the expression vector. The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed by the vector. Therefore, it will be necessary to select for transformed host cells. One selection technique involves incorporating into the expression vector a nucleic acid sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance. Alternatively, the gene for such selectable trait can be on another vector, which is used to co-transform the desired host cell.

Host cells that have been transformed by the recombinant nucleic acid of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings

disclosed herein to permit the expression of the polypeptide, which can then be recovered.

Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae* and *Pichia pastoris*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells (for example COS-1, COS-7, CHO, NIH 3T3, NS0 and BHK cells) and insect cells (for example Drosophila, SF9 cells).

Those vectors that include a replicon such as a procaryotic replicon can also include an appropriate promoter such as a procaryotic promoter capable of directing the expression (transcription and translation) of the genes in a bacterial host cell, such as *E. coli*, transformed therewith.

A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention.

Typical procaryotic vector plasmids are pUC18, pUC19, pBR322 and pBR329 (available from Biorad Laboratories, Richmond, CA, USA), p*Trc*99A and pKK223-3 (available from Pharmacia Piscataway, NJ, USA) and the pET system (T7 promoter, Novagen Ltd).

A typical mammalian cell vector plasmid is pSVL available from Pharmacia, Piscataway, NJ, USA. This vector uses the SV40 late promoter to drive expression of cloned genes, the highest level of

expression being found in T antigen-producing cells, such as COS-1 cells.

An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. This vector uses the glucocorticoid-inducible promoter of the mouse mammary tumour virus long terminal repeat to drive expression of the cloned gene.

Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers *his3, trp1, leu2* and *ura3*. Plasmids pRS413-416 are Yeast Centromere plasmids (YCps).

Further useful vectors for transformation of yeast cells, such as *Pichia*, include the 2μ plasmid pYX243 (available from R and D Systems Limited) and the integrating vector pPICZ series (available from Invitrogen).

A variety of methods have been developed to operatively link DNA to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion as described

earlier, is treated with bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, enzymes that remove protruding, 3'-single-stranded termini with their 3'-5'-exonucleolytic activities. and fill in recessed 3'-ends with their polymerizing activities.

The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CN, USA.

A desirable way to modify the nucleic acid encoding the compound of the invention or a portion thereof is to use the polymerase chain reaction as disclosed by Saiki *et al* (1988) *Science* **239**, 487-491.

In this method the nucleic acid to be enzymatically amplified is flanked by two specific oligonucleotide primers which themselves become incorporated into the amplified nucleic acid. The said specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

Exemplary genera of yeast contemplated to be useful in the practice of the present invention are *Pichia, Saccharomyces, Kluyveromyces, Candida, Torulopsis, Hansenula, Schizosaccharomyces, Citeromyces, Pachysolen, Debaromyces, Metschunikowia, Rhodosporidium, Leucosporidium, Botryoascus, Sporidiobolus, Endomycopsis*, and the like. Preferred genera are those selected from the group consisting of *Pichia, Saccharomyces, Kluyveromyces, Yarrowia* and *Hansenula*. Examples of *Saccharomyces* are *Saccharomyces cerevisiae*, *Saccharomyces italicus* and *Saccharomyces rouxii*. Examples of *Kluyveromyces* are *Kluyveromyces fragilis* and *Kluyveromyces lactis*. Examples of *Hansenula* are *Hansenula polymorpha, Hansenula anomala* and *Hansenula capsulata*. *Yarrowia lipolytica* is an example of a suitable *Yarrowia* species.

Methods for the transformation of *S. cerevisiae* are taught generally in EP 251 744, EP 258 067 and WO 90/01063, all of which are incorporated herein by reference.

Suitable promoters for *S. cerevisiae* include those associated with the *PGK1* gene, *GAL1* or *GAL10* genes, *CYC1*, *PHO5*, *TRP1*, *ADH1*, *ADH2*, the genes for glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, triose phosphate isomerase, phosphoglucose isomerase, glucokinase, α-mating factor pheromone, a-mating factor pheromone, the *PRB1* promoter, the *GUT2* promoter, and hybrid promoters involving hybrids of parts of 5' regulatory regions with parts of 5' regulatory regions of other promoters or with upstream activation sites (eg the promoter of EP-A-258 067).

The transcription termination signal is preferably the 3' flanking sequence of a eukaryotic gene which contains proper signals for transcription termination and polyadenylation. Suitable 3' flanking sequences may, for example, be those of the gene naturally linked to the expression control sequence used, i.e. may correspond to the promoter. Alternatively, they may be different in which case the termination signal of the *S. cerevisiae AHD1* gene is preferred.

The present invention also relates to a host cell transformed with a polynucleotide vector construct of the present invention. The host cell can be either procaryotic or eukaryotic. Bacterial cells are preferred procaryotic host cells and typically are a strain of *E. coli* such as, for example, the *E. coli* strains DH5 available from Bethesda Research Laboratories Inc., Bethesda, MD, USA, and RR1 available from the American Type Culture Collection (ATCC) of Rockville, MD, USA (No ATCC 31343). Preferred eukaryotic host cells include yeast and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic cell line. Preferred eukaryotic host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658 and monkey kidney-derived COS-1 cells available from the ATCC as CRL 1650 or WSØ cells.

Transformation of appropriate cell hosts with a nucleic acid constructs of the present invention is accomplished by well known methods that typically depend on the type of vector used. With regard to transformation of procaryotic host cells, see, for example, Cohen *et al. Proc. Natl. Acad. Sci. USA*, **69**: 2110 (1972); and Sambrook *et al*,

Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989). Transformation of yeast cells is described in Sherman *et al*, *Methods In Yeast Genetics, A Laboratory Manual*, Cold Spring Harbor, NY (1986). The method of Beggs. *Nature*. **275**: 104-109 (1978) is also useful. With regard to vertebrate cells, reagents useful in transfecting such cells, for example calcium phosphate and DEAE-dextran or liposome formulations, are available from Stratagene Cloning Systems, or Life Technologies Inc, Gaithersburg, MD 20877, USA.

Successfully transformed cells, *i.e.* cells that contain a nucleic acid construct of the present invention. can be identified by well known techniques. For example. cells resulting from the introduction of an expression construct of the present invention can be grown to produce the polypeptide of the invention. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern. *J. Mol. Biol.*, **98**: 503 (1975) or Berent *et al*, *Biotech.*, **3**: 208 (1985). Alternatively, the presence of the protein in the supernatant can be detected using antibodies as described below.

In addition to directly assaying for the presence of recombinant nucleic acid, successful transformation can be confirmed by well known immunological methods when the recombinant nucleic acid is capable of directing the expression of the protein. For example, cells successfully transformed with an expression vector produce proteins displaying appropriate antigenicity. Samples of cells suspected of being transformed are harvested and assayed for the protein using suitable antibodies.

Thus, in addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium. Preferably, the culture also contains the protein.

Nutrient media useful for culturing transformed host cells are well known in the art and can be obtained from several commercial sources.

A third aspect of the invention provides a vector comprising a nucleic acid according to the second aspect of the invention.

A fourth aspect of the invention provides a host cell comprising a vector according to the third aspect of the invention.

Preferably, the host cell is a mammalian cell.

More preferably the host cell is NS0.

A fifth aspect of the invention provides a pharmaceutical composition comprising a compound according to the first aspect of the invention and a pharmaceutically acceptable carrier.

The compounds and compositions of the invention are administered in any suitable way, usually parenterally, for example intravenously, intraperitoneally or, preferably (for bladder cancer), intravesically (*i.e.* into the bladder), in standard sterile, non-pyrogenic formulations of diluents and carriers, for example isotonic saline (when administered

intravenously).

A sixth aspect of the invention provides a compound according to the first aspect of the invention for use in medicine.

The compounds and compositions of the invention may be used to treat a patient with any disease involving a dysfunction of a population of cells expressing PEM, said compounds and compositions selectively targeting and destroying said population of cells within a patient. For example, said compounds and compositions may be used in the treatment of cancer, *e.g.* cancer of the breast, ovaries, lung, stomach, intestines, blood *etc*. Thus, anti-tumour cell antigen antibodies can be used to deliver a cytotoxic portion with endonuclease activity to a tumour cell. Antibodies that are internalised upon contact with the target antigen are used, such that the cytotoxic portion enters the cytosol of the tumour cell, where it can trigger cell death.

In principle, the compounds and compositions of the invention may be used to treat any mammal, including pets such as dogs and cats and agriculturally important animals such as cows, horses, sheep and pigs.

Preferably, the patient is human.

A seventh aspect of the invention provides the use of a compound according to first aspect of the invention in the preparation of a medicament for treating a mammal having said target cells to be destroyed.

Preferably, the medicament is for treating cancer, such as ovarian cancer.

A eighth aspect of the invention provides a method of treating a mammal having target cells to be destroyed, the method comprising administering a compound according to the first aspect of the invention to said mammal.

In a preferred embodiment of the seventh and eighth aspects of the invention, the mammal is a human.

Preferably, the target cells to be destroyed are cancer cells. More preferably, the cancer cells are epithelial cancer cells, such as ovarian, gastric, colorectal and/or pancreatic cancer cells. Most preferably, the cancer cells are ovarian cancer cells.

The invention will now be described in detail with reference to the following figures and examples:

Figure 1 shows the complete coding sequence of human DNAse I.

Figure 2 shows the DNAse I sequence used in the exemplary constructs (*e.g.* pAS41).

Figure 3 shows the HMFG1 light chain insert (A) and heavy chain insert (B) used in the exemplary constructs (*e.g.* pAS6).

Figure 4 shows the linker and hinge-linker oligonucleotides used in (A) the whole antibody-DNase and (B) the Fab'2-DNase exemplary constructs. Note, in Figure 4(A) a deletion of one or more codons

between the HMFG1 hinge and the linker is represented as ΔG.

Figure 5 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS23 (linker sequence underlined).

Figure 6 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS27 (linker sequence and NLS underlined).

Figure 7 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS34 (linker sequence underlined).

Figure 8 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS35 (linker sequence underlined). The lower case 'g' represents a silent mutation caused by PCR amplification.

Figure 9 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS36 (linker sequence underlined). The lower case 'c' represents a silent mutation caused by PCR amplification.

Figure 10 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS37 (linker sequence and NLS underlined).

Figure 11 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS38 (linker sequence and NLS

underlined). The lower case 'g' represents a silent mutation caused by PCR amplification.

Figure 12 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS39 (linker sequence underlined). The lower case 'c' represents a silent mutation caused by PCR amplification.

Figure 13 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS101 (linker sequence underlined).

Figure 14 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS102 (hybrid hinge and linker sequence underlined).

Figure 15 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS103 (hybrid hinge and linker sequence underlined).

Figure 16 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS104 (hybrid hinge and linker sequence underlined, mutations (compared to pAS103) at positions 775 and 924 are shaded).

Figure 17 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS105 (linker sequence and NLS underlined).

Figure 18 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS106 (hybrid hinge + linker sequence and NLS are underlined).

Figure 19 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS107 (hybrid hinge + linker sequence and NLS are underlined).

Figure 20 shows a schematic diagram of the pEE6 expression vector used in the exemplary constructs.

Figure 21 shows autoradiographs from immuno-precipitation experiments with metabolically labelled transient transfectants:

GEL A

Lane 1 shows the precipitation of supernatant from mock-transfected cells.

Lane 2 is from cells transfected with hHMFG-1 (construct 6) giving expected molecular weights of about 51.2 and 26.4 kDa for the heavy and light chains, respectively.

Lane 3 shows construct 34 antibody construct which has human DNase I fused to the C-terminus of the heavy chain gene. As expected, the size of the heavy chain gene has increased to about 80.7 kDa.

Samples from whole antibody DNase I constructs 35, 36 and 39 were run on the gel (Lanes 4 to 6) but were not sufficiently well expressed to be visible, in this experiment.

In subsequent experiments using this method, construct 39 was detectable but weak, and constructs 35 and 36 were detectable but very weak. Constructs 37 and 38 have not been tested in this assay system.

Lanes 8 to 10 are fusion of humanised HMFG1 $F(ab')_2$ with human DNase I (constructs 41, 23 and 102, respectively). $F(ab')_2$ alone was included in this set of experiments (lane 7, construct 41) but did not express, this was included in later experiments (see gels C and D).

In addition to the light chain (about 26.4 kDa) and the Fd-DNAse I fusion (about 56.6 kDa), a third major band is observed at around 40 kDa. Interestingly, this band is observed in the humanised HMFG-1 fusions but not in the antibody alone. Since an anti-$F(ab')_2$ antibody was used for immuno-precipitation, it is unlikely that this can be proteolysis between immunoglobulin and DNase I sequence. It probably represents a population of polypeptide produced by premature transcriptional termination (due to DNase I sequence in the 3'-end of the fusion mRNA).

GEL B

This is the non-reducing gel counterpart to gel A, described above. Lane 1 is the mock-transfected control cells and lanes 2 and 3 are from the cells transfected with humanised HMFG1 alone (construct 6) and the humanised HMFG-1 fused at the C-terminus to human DNase I, respectively. As before, lanes 4 to 6 are from cell supernatants from cells transfected with constructs 35, 36 and 39. The gel shows that both the whole antibody and the antibody-DNase I fusion are assembled, with the DNase fusion giving a higher molecular weight compared to the antibody alone.

Figure 22 shows a typical standard curve used to determine the concentration of PDTRP-binding material in the supernatants of transiently transfected L761h cells. Each point on the curve has been determined twice.

Figure 23 shows typical standard curves used to determine the concentration of bovine DNAse I.

Figure 24 shows corrected DNase I activity in transiently expressed humanised HMFG1 whole antibody-human DNAse I fusions (*i.e.* pAS34, pAS34, pAS35 and pAS6[control]).

Figure 25 shows the corrected DNAse I activity in transiently expressed humanised HMFG1 $F(ab')_2$-human DNase I fusions (*i.e.* pAS101, pAS102, pAS103 and pAS41[control]).

Figure 26 shows results of the cytotoxicity assay.

Figure 27 shows the % of MCF7 cells killed afetr incubation with the exemplary constructs.

EXAMPLES

(A) Mammalian expression of humanised HMFG1-DNase constructs

The human HMFG1 light and heavy chain (with or without engineering a fusion to human DNase I), were cloned into the pEE6 expression vector system for expression in mammalian CHO or myeloid NS0 cells (see figure 20). The vector system was originally developed by Celltech Ltd (UK) and is now owned by al-Lonza (see Young & Owens, 1994, *J. Immunol. Meth.* **168**:149-165). The vector consists of two human cytomegalovirus promoters (hCMV) for both the heavy and light chain genes. Each transcription unit is completed by the poly-adenylation signal (pA) with an optional immunoglobulin terminator sequence (Ig term.) located between the heavy and light chain transcription units. Propagation in *E.coli* can be selected for by the presence on an ampicillin resistance gene (not shown in Fig 20). The inclusion of a glutamine synthetase gene (GS) in the vector allows the stable NS0 transfectomas to be selected by growth in glutamine free media, since NS0 cells are GS0 and cannot otherwise grow in glutamine free media.

Exemplary humanized HMFG1-DNAse I fusion constructs of the invention are detailed in figures 5 to 19.

(B) Immuno-precipitation of metabolically labelled transient transfectants

CHO-L761h cells (Cockett *et al.*, 1990, *Nuc. Acids Res.* **19**:319-325) were transfected, according to the modification of Gorman et al, 1985),

with expression vectors containing either whole HMFG1 antibody or $F(ab')_2$ fragment of the antibody along with the various fusion constructs of their respective heavy chains and human DNase I. The cells were then incubated with either 50 μCi ^{35}S methione for 72 h in methionine-free medium. Secreted product was precipitated with a rabbit anti-human $F(ab')_2$ antibody bound to protein A Sepharose. Bound material was eluted in either reducing or non-reducing SDS-PAGE loading buffer and run on gels. The autoradiographs (see Figure 21) above were generated from those gels after drying them.

(C) <u>Estimation of the efficiency of DNase constructs in supernatants</u>

Introduction

This set of experiments was designed to standardise the amount of construct in a given DNase I activity assay and to allow us to comment on the amount of activity a particular construct possesses. Given that the antibody-DNase I fusions are so different to the $F(ab')_2$-DNase I fusions it is best not to compare the two groups. Once we have purified the protein, we will have a better idea of the exact molecular configuration of all species. Then, and only then, will it be sensible to compare amongst groups.

Determination of concentration of constructs

The concentration of constructs in supernatants from transiently transfected L761H cells was determined in a PDTRP-binding ELISA. To each well of a Maxisorb 96-well ELISA plate (Nunc) was added 100 μl of

carbonate buffer containing 100 ng of recombinant GST-(PDTRP)$_7$ fusion protein (Gendler *et al.*, 1990, *J. Mol. Biol.* **265**:15286-93). After overnight binding at 4°C, the plate was washed three times in PBS-Tween (*i.e.* PBS containing 0.05% Tween-20). The plate was then blocked with three 3-minute washes of PBS-Tween containing 1% BSA.

For each construct, 100 µl of supernatant was added to a well on the plate. In addition, hHMFG-1 of known concentration was serially diluted down the plate using doubling dilutions in 100 µl of PBS-Tween per well. The plate was incubated for a further 1 h at 30°C, then 200 ng of MC135 anti-human kappa light chain antibody (binding site) in 100 µl of PBS-Tween was added to each well for 1 h at 30°C. After three 3-minute washes in PBS-Tween, 100 µl of anti-mouse IgG-peroxidase conjugate (Jackson 315-035-045), diluted 1:2000 in PBS-Tween, was added to each well and incubated for 1 h at 30°C. Following a final set of three 3-minute washes in PBS-Tween, 100 µl of TMB substrate (Sigma) was added to each well of the plate and, after a colour developed, the optical density at 630 nm of the solution in each well of the plate was determined.

Results

(see Figure 22)

(D) <u>Corrected bovine DNase I standard curves and DNase assay</u>

DNase activity was determined using a modification of the methyl green-DNA complex degradation method (Sinicropi *et al.*, 1994, *Analyt. Biochem.* **222**:351-358). Briefly, a 1:1 solution of the assay buffer and

methyl green-salmon sperm DNA complex was mixed together to give a total volume of 0.2 ml. To this, 0.1 ml of tissue culture supernatant from transiently transfected CHO-L761h cells was added and the mixture incubated at 37°C. DNA cleavage by DNase results in a reduction in absorbance at 620 nm. Figure 23 shows a standard curve produced with various concentrations of bovine DNase I over a number a time point.

Figures 24 and 25 show DNAse activity for the whole HMFG1 antibody- and $F(ab')_2$ - DNase fusions, respectively.

(E) <u>Cytotoxicity of DNAse constructs</u>

Method

DNase constructs were transfected into CHO L761h cells using a calcium phosphate co-precipitation method (Gorman *et al.*, 1985, In: *DNA cloning* (2nd edition), Glover A(ed.), Academic Press, NY, 163-188). Included in the experiment were negative controls, consisting of cells transfected with TE buffer alone or with TE buffer and pEE6 expression vector. In addition to these controls, vectors that express hHMFG-1 (pAS6) and $F(ab')_2$ of hHMFG1 (both with specificity for PEM but without DNase I) were included.

The supernatant from these cells was harvested after 72 h of expression, followed by centrifugation to remove dead cells. MCF-7 cells were incubated for 1 h at 37°C with an aliquot of each of these supernatants. The amount of cellular lactate dehydrogenase (LDH) released from the MCF-7 cells due to the cytotoxicity of the supernatant was determined

using the CytoTox96 cytotoxic assay kit (Promega). Total lysis ('total LDH') was determined by measuring the target cell maximum LDH release using the kits lysis solution. The percentage of cells killed was then calculated as the proportion of the LDH released to the total LDH released. For each construct, the cytotoxicity assay was performed in quadruplicate, except for assay of pAS38 and 39, which were performed in triplicate. The values of LDH release for each construct were compared against either $F(ab')_2$ or whole antibody, or each other, using a one-tailed t-test in Excel.

Results

Figures 26 and 27 shows that there is negligible cell killing with either pAS6 (HMFG1 alone) or with pAS41 ($F(ab')_2$ alone). All of the hHMFG1 $F(ab')_2$-DNase I constructs kill significantly more cells than the $F(ab')_2$ fragment alone ($p<0.00193$) and all of the antibody-DNase I constructs kill significantly more cells than antibody alone ($p<0.00783$), except for perhaps pAS34 ($p<0.021$).

(F) <u>Use of the DNase-I/huHMFG-1 Fab fusion protein in the treatment of ovarian cancer</u>

Patients diagnosed with ovarian cancer are treated by intravenous injection of the DNaseI/huHMFG-1 Fab fusion protein. Typically, a dose of between 1 to 100 mg will be administered weekly.

Therapeutic response is measured by the normal clinical procedures that are well known in the art, for example radio-imaging methods.

CLAIMS

1. A compound comprising a target cell-specific portion and a cytotoxic portion characterised in that:

(i) the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof; and

(ii) the cytotoxic portion has endonucleolytic activity.

2. A compound according to Claim 1 wherein the target cell-specific portion comprises an humanised HMFG-1 antibody or an antigen binding fragment thereof.

3. A compound according to Claim 2 wherein the target cell-specific portion is an humanised HMFG-1 antibody.

4. A compound according to Claim 1 or 2 wherein the target cell-specific portion comprises an antigen binding fragment of the humanised antibody selected from the group consisting of Fab-like molecules, such as Fab and $F(ab')_2$, Fv molecules, disulphide-linked Fv molecules, ScFv molecules and single domain antibodies (dAbs).

5. A compound according to Claim 4 wherein the target cell-specific portion comprises a Fab molecule.

6. A compound according to Claim 4 wherein the target cell-specific

portion comprises a $F(ab')_2$ molecule.

7. A compound according to Claim 1 wherein the target cell-specific portion comprises at last part of one or both of the amino acid sequences of Figure 3.

8. A compound according to Claim 7 wherein the target cell-specific portion comprises both of the amino acid sequences of Figure 3.

9. A compound according to any one of Claims 1 to 8 wherein the cytotoxic portion has DNA endonucleolytic activity.

10. A compound according to Claim 9 wherein the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease.

11. A compound according to Claim 10 wherein the endonuclease is a mammalian deoxyribonuclease I.

12. A compound according to Claim 11 wherein the endonuclease is a human deoxyribonuclease I.

13. A compound according to Claim 1 wherein the endonuclease is a restriction endonuclease.

14. A compound according to Claim 10 wherein the cytotoxic portion comprises the amino acid sequence of Figure 2.

15. A compound according to any one of Claims 1 to 14 wherein a nuclear

localization signal is incorporated.

16. A compound according to Claim 15 wherein the nuclear localization signal comprises the sequence PKKKRKV.

17. A compound according to any one of Claims 1 to 16 wherein the target cell-specific portion and the cytotoxic portion are fused.

18. A compound according to Claim 17 wherein the target cell-specific portion and the cytotoxic portion are separated by a lniker sequence.

19. A compound according to Claim 18 wherein the linker sequence is or comprises GG or GSGG.

20. A nucleic acid encoding a compound as defined in any one of Claims 17 to 19.

21. A vector comprising a nucleic acid according to Claim 20.

22. A host cell comprising a vector according to Claim 21.

23. A pharmaceutical composition comprising a compound according to any one of Claims 1 to 19 and a pharmaceutically acceptable carrier.

24. A compound according to any one of Claims 1 to 19 for use in medicine.

25. Use of a compound according to any one of Claims 1 to 19 in the

preparation of a medicament for treating a mammal having said target cells to be destroyed.

26. A method of treating a mammal having target cells to be destroyed, the method comprising administering a compound according to any one of Claims 1 to 19 to said mammal.

27. A use according to Claim 25 or a method according to Claim 26 wherein the mammal is a human.

27. A use according to Claim 25 or a method according to Claim 26 wherein the target cells to be destroyed are cancer cells.

28. A use or a method according to Claim 27 wherein the cancer cells are epithelial cancer cells.

29. A use or a method according to Claim 28 wherein the cancer cells are ovarian, gastric, colorectal and/or pancreatic cancer cells.

30. A use or a method according to Claim 29 wherein the cancer cells are ovarian cancer cells.

31. A compound substantially as described herein, preferably with reference to one or more of the accompanying figures.

ABSTRACT

Compounds for Targeting

A compound comprising a target cell-specific portion and a cytotoxic portion characterised in that the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof, and the cytotoxic portion has endonucleolytic activity. Preferably, the target cell-specific portion comprises an humanised HMFG-1 antibody or an antigen binding fragment thereof. Advantageously, the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease, *e.g.* a human DNA endonuclease I. The invention further provides nucleic acids encoding the compounds of the invention, and the use of such compounds in medicine, *e.g.* in the treatment of cancer.

Figure No.
no figure.

5|9|00

FIGURE 1

Human DNase I (complete mRNA)

```
LOCUS       HUMDNASEI     1039 bp    mRNA            PRI       06-MAR-1995
DEFINITION  Human DNase I mRNA, complete cds.
ACCESSION   M55983
VERSION     M55983.1  GI:181623
KEYWORDS    DNase I.
SOURCE      Human pancreus, cDNA to mRNA.
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
            Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 1039)
  AUTHORS   Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.
  TITLE     Recombinant human DNase I reduces the viscosity of cystic fibrosis
            sputum
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  MEDLINE   91067672
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                     /gene="DNase I"
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                     DAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQWLI
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                     /gene="DNase I"
     mat peptide     226..1005
                     /gene="DNase I"
                     /product="DNase I"
BASE COUNT       226 a    305 c    282 g    226 t
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       61 ttttctttaa gcagcaaaag gagaaaattg tcatcaaagg atattccaga ttcttgacag
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      661 gctctctatg acgtctacct ggatgtccaa gagaaatggg gcttggagga cgtcatgttg
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      841 cccacgcact gtgcctatga caggatcgtg gttgcaggga tgctgctccg aggcgccgtt
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 961 gcccaagcca tcagtgacca ctatccagtg gaggtgatgc tgaagtgagc agcccctccc
1021 cacaccagtt gaactgcag
//
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FIGURE 2 human DNase I construct

```
LOCUS       HUMDNASE.DN     753 bp    mRNA            PRI       06-MAR-1995
DEFINITION  Human DNase I mRNA, complete cds, Mature sequence modified to remove NarI site
ACCESSION   M55983
NID         g181623
KEYWORDS    DNase I.
SOURCE      Human pancreas, cDNA to mRNA.
  ORGANISM  Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 1039)
  AUTHORS   Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.
  TITLE     Recombinant human DNase I reduces the viscosity of cystic fibrosis
            sputum
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  MEDLINE   91067672
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                     /tissue_type="pancreas"
     sig_peptide     160. .225
                     /gene="DNase I"
     CDS             160. .1008
                     /gene="DNase I"
                     /codon_start=1
                     /product="DNase I"
                     /db_xref="PID:g181624"
                     /translation="LKIAAFNIQTFGETKMSNATLVSYIVQILSRYDIALVQEVRDSH
                     LTAVGKLLDNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSAVDSYYYDDGCE
                     PCGNDTFNREPAIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALYDVYLDVQEKWGL
                     EDVMLMGDFNACCSYVRPSQWSSIRLWTSPTFQWLIPDSADTTATPTHCAYDRIVVAG
                     MLLRGAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVMLK"
     gene            160. .1008
                     /gene="DNase I"
     mat_peptide     226. .1005
                     /gene="DNase I"
                     /product="DNase I"
BASE COUNT      168 a    236 c    220 g    159 t
ORIGIN

        1 CTGAAGATCG CAGCCTTCAA CATCCAGACA TTTGGGGAGA CCAAGATGTC CAATGCCACC
       61 CTCGTCAGCT ACATTGTGCA GATCCTGAGC CGCTACGACA TCGCCCTGGT CCAGGAGGTC
      121 AGAGACAGCC ACCTGACTGC CGTGGGGAAG CTGCTGGACA ACCTCAATCA GGACGCACCA
      181 GACACCTATC ACTACGTGGT CAGTGAGCCA CTGGGACGGA ACAGCTATAA GGAGCGCTAC
      241 CTGTTCGTGT ACAGGCCTGA CCAGGTGTCT GCGGTGGACA GCTACTACTA CGATGATGGC
      301 TGCGAGCCCT GCGGGAACGA CACCTTCAAC CGAGAGCCAG CCATTGTCAG GTTCTTCTCC
      361 CGGTTCACAC AGGTCAGGGA GTTTGCCATT GTTCCCCTGC ATCCCCCCCC CGGGGACGCA
      421 CTACCCCAGA TCGACGCTCT CTATGACGTC TACCTGGATG TCCAAGAGAA ATGGGGCTTG
      481 GAGGACGTCA TGTTGATGGG CGACTTCAAT GCGGGCTGCA GCTATGTGAC ACCCTCCCAG
      541 TGGTCATCCA TCCGCCTGTG GACAAGCCCC ACCTTCCAGT GGCTGATCCC CGACAGCGCT
      601 GACACCACAG CTACACCCAC GCACTGTGCC TATGACAGGA TCGTGGTTGC AGGGATGCTG
      661 CTCCGAGGCC CCCTTGTTCC CGACTCGGCT CTTCCCTTTA ACTTCCAGGC TGCCTATGGC
      721 CTGAGTGACC AACTGGCCCA AGCCATCAGT GACCACTATC CAGTGGAGGT CATGCTGAAG
      781 TGA
//
```

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FIGURE 3

(A) pAS6 - light chain

LOCUS HMFG1LC2.D 721 bp DNA
DEFINITION HUMANISED HMFG1 LIGHT CHAIN VnP LEADER.
ACCESSION
KEYWORDS
SOURCE
ORGANISM
REFERENCE 1 (BASES 1 TO 342)
AUTHORS VERHOEYEN ET AL
TITLE CONSTRUCTION OF RESHAPED HMFG1 ETC
JOURNAL IMMUNOL. (1993):78, 364-370
COMMENT SCANNED IN FROM JOURNAL
FEATURES
SITES

This is the sequence of the HMFG1 light chain gene with the Vnp leader sequence attached. Translate from residue 1. Note residue 399 is T > A in all clones leading to R133 silent mutation (T in Verhoeyen paper)

BASE COUNT 197 a 202 c 182 g 140 t
ORIGIN (Leader ?)

```
     1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCGAC
    61 ATCCAGATGA CCCAGAGCCC AAGCAGCCTG AGCGCCAGCG TGGGTGACAG AGTCACCATC
   121 ACCTGTAAGT CCAGTCAGAG CCTTTTATAT ACTAGCAATC AAAAGATCTA CTTGGCCTGG
   181 TACCAGCAGA AGCCAGGTAA GGCTCCAAAG CTGCTGATCT ACTGGGCATC CACTAGGGAA
   241 TCTGGTGTGC CAAGCAGATT CAGCGGTAGC GGTAGCGGTA CGGACTTCAC CTTCACCATC
   301 AGCAGCCTCC AGCCAGAGGA CATCGCCACC TACTACTGCC AGCAATATTA TAGATATCCT
   361 CGGACGTTCG GCCAAGGGAC CAAGGTCCAA ATCAAACGAA CTGTGGCTGC ACCATCTGTC
   421 TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG
   481 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA
   541 TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA AGGACAGCAC CTACAGCCTC
   601 AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA CGCCTGCGAA
   661 GTCACCCATC AGGGCCTGAG CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAG
   721 A
//
```

FIGURE 3

(B) pA86 - heavy chain

```
LOCUS       HHMFG1HC.D   1404 bp    DNA
DEFINITION  HUMANISED HMFG1 heavy chain
ACCESSION   HHMFG1H
KEYWORDS
SOURCE
  ORGANISM
REFERENCE
  AUTHORS   VERHOEYEN ET AL
  TITLE     CONSTRUCTION OF RESHAPED HMFG1 etc
  JOURNAL   IMMUNOL. (1993):78, 364-370
COMMENT     VH domain SCANNED IN FROM JOURNAL
FEATURES    AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES    Residue 963 is C > T leading to silent mutation in all clones
  SITES     Note
BASE COUNT      333 a    439 c    379 g    253 t
ORIGIN          ?
```

LEADER.

```
   1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
  61 GTGCAGCTCC TGCACTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGGGTAA ATGA
```

ANTIBODY DNAse FUSIONS made HERE (34→39.)

End of lower hinge region of HEAVY CHAIN. PAPE AMINO ACID SEQ. Fab'_2 Fusions were Made at this point.

Those with HYBRID HINGES ARE ALTERED FURTHER UP i.e.

THIS PART GACAAAACTGACACA
is → D K T H T

AFTER THIS SEQUENCE YOU GET THE HYBRID HINGE + LINKER SEQUENCES Then DNAse I

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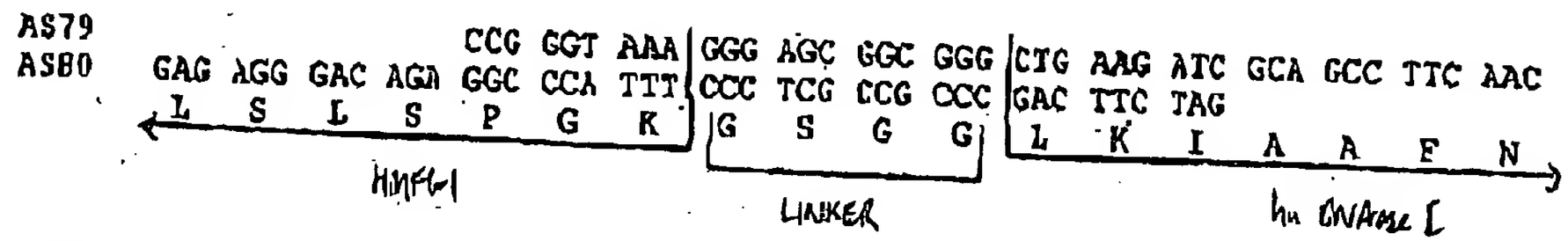
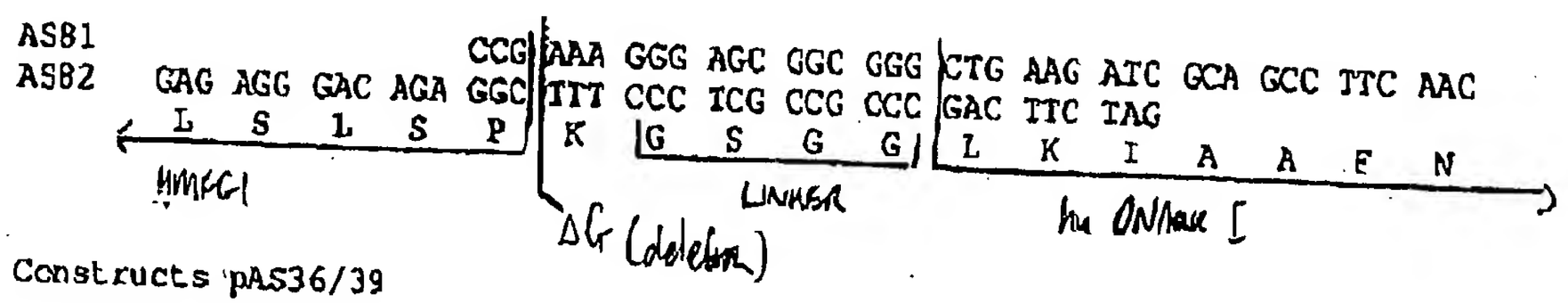
FIGURE 4

(A.) Oligos involved in the fusion of whole antibody-DNase

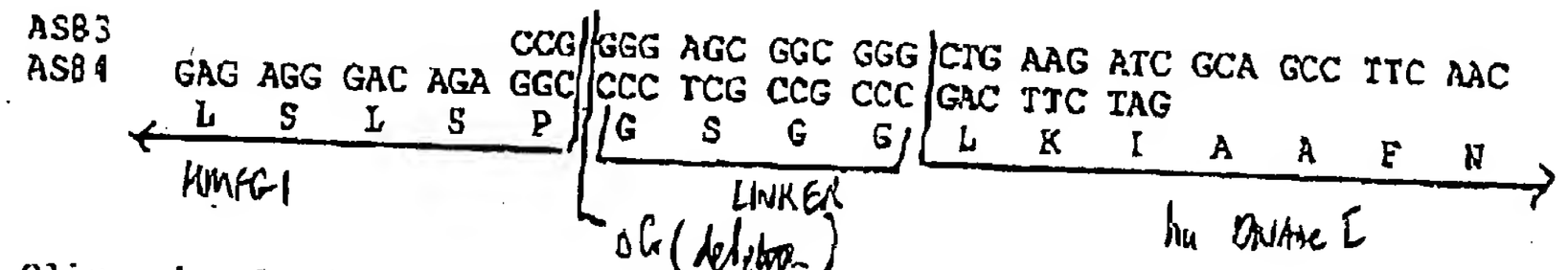
Constructs pAS34/37



Constructs pAS35/38

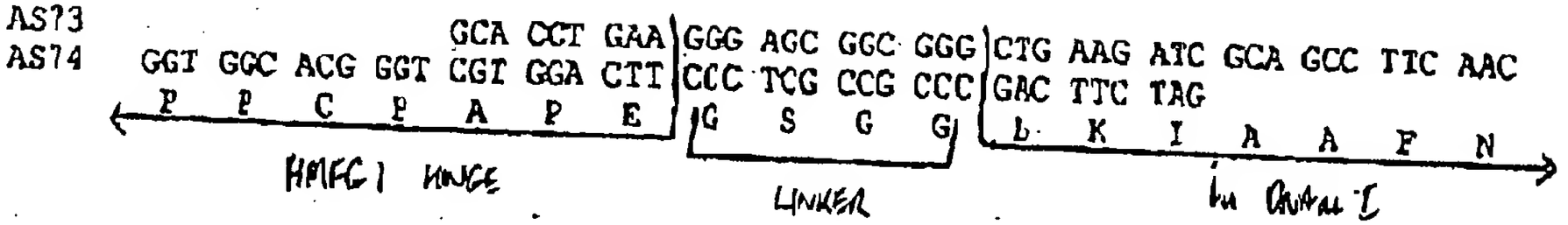


Constructs pAS36/39



Oligos involved in the fusion of Fab'2-DNaseI

Constructs pAS23/27



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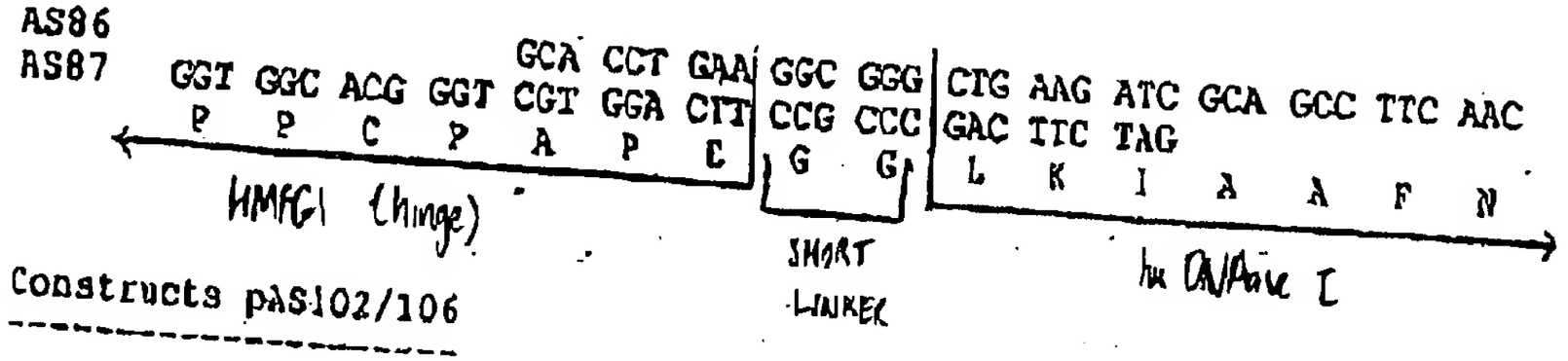
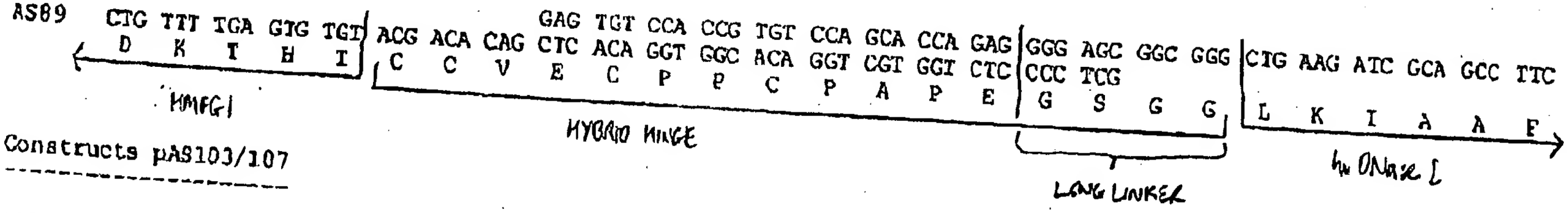
(b) Oligos involved in the fusion of new Fab'2-DNaseI molecules (5.7.99)

Constructs pAS101/105



Constructs pAS102/106



Constructs pAS103/107

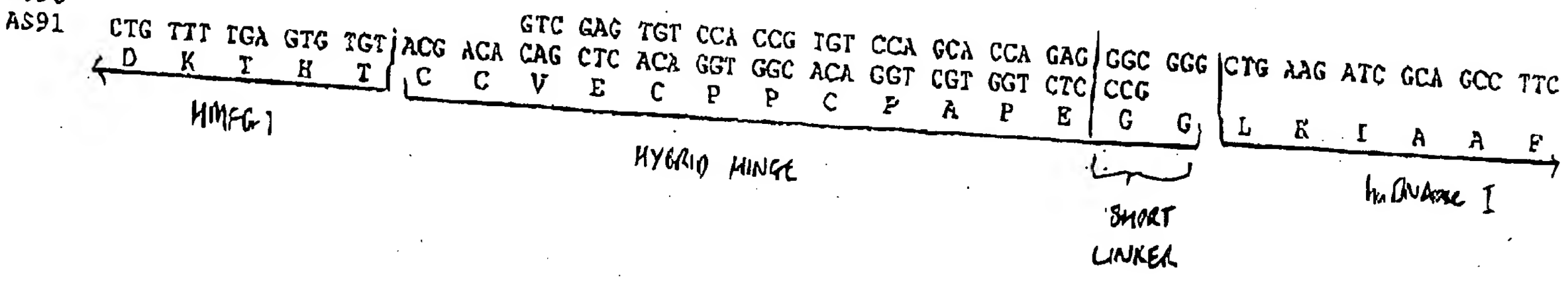


FIGURE 4

FIGURE 5

(A) **pAS23**

LOCUS PAS23.DNA 1554 bp mRNA PRI 06-MAR-1995
DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (construct 1)
ACCESSION
NID
KEYWORDS DNase I.
SOURCE DNase I sequence is from assembled oligos (thus modified c/f MHDNASE1.dna)
ORGANISM Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.
TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
MEDLINE 91067672
BASE COUNT 344 a 468 c 434 g 308 t
ORIGIN

```
   1 ATGGCATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
  61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GGAGCGGCGG GCTGAAGATC
 781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC
 841 TACATTGTGC AGATCCTGAG CCGCTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC
 901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT
 961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCGTG
1021 TACAGGCCTG ACCAGGTGTC TGCGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC
1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCTTCTC CCGGTTCACA
1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCCTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG
1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGGACGTC
1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC
1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA
1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGGTTG CAGGGATGCT GCTCCGAGGG
1441 GCCGTTGTTC CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC
1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCAGTGGAGG TGATGCTGAA GTGA
```

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File : PAS23.DNA
Range : 1 - 1554 Mode : Normal
Codon Table : Universal

FIGURE 5(B)

```
                9                  18                  27                  36                  45                  54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63                  72                  81                  90                  99                 108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117                 126                 135                 144                 153                 162
    CTC AAC CTC TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171                 180                 189                 198                 207                 216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225                 234                 243                 252                 261                 270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279                 288                 297                 306                 315                 324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333                 342                 351                 360                 369                 378
    GAC ACA CCC CTC TAT TAC TCT CCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387                 396                 405                 414                 423                 432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441                 450                 459                 468                 477                 486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495                 504                 513                 522                 531                 540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549                 558                 567                 576                 585                 594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G
```


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```
                603                 612                 621                 630                 639                 648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

                657                 666                 675                 684                 693                 702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

                711                 720                 729                 738                 747                 756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

                765                 774                 783                 792                 801                 810
GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   G   S   G   G   L   K   I   A   A   F   N   I   Q   T   F   G   E

                819                 828                 837                 846                 855                 864
ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   K   M   S   N   A   T   L   V   S   Y   I   V   Q   I   L   S   R

                873                 882                 891                 900                 909                 918
TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   D   I   A   L   V   Q   E   V   R   D   S   H   L   T   A   V   G

                927                 936                 945                 954                 963                 972
AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   L   L   D   N   L   N   Q   D   A   P   D   T   Y   H   Y   V   V

                981                 990                 999                1008                1017                1026
AGT GAG CCA CTC CCA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   E   P   L   G   R   N   S   Y   K   E   R   Y   L   F   V   Y   R

               1035                1044                1053                1062                1071                1080
CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   D   Q   V   S   A   V   D   S   Y   Y   Y   D   D   G   C   E   P

               1089                1098                1107                1116                1125                1134
TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   G   N   D   T   F   N   R   E   P   A   I   V   R   F   F   S   R

               1143                1152                1161                1170                1179                1188
TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   T   E   V   R   E   F   A   I   V   P   L   H   A   A   P   G   D

               1197                1206                1215                1224                1233                1242
```


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```
GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   V   A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q   E   K

        1251        1260        1269        1278        1287        1296
TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 W   G   L   E   D   V   M   L   M   G   D   F   N   A   G   C   S   Y

        1305        1314        1323        1332        1341        1350
GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   R   P   S   Q   W   S   S   I   R   L   W   T   S   P   T   F   Q

        1359        1368        1377        1386        1395        1404
TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TCT GCC TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 W   L   I   P   D   S   A   D   T   T   A   T   P   T   H   C   A   Y

        1413        1422        1431        1440        1449        1458
GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   R   I   V   V   A   G   M   L   L   R   G   A   V   V   P   D   S

        1467        1476        1485        1494        1503        1512
GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   L   P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q

        1521        1530        1539        1548
GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'
--- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   I   S   D   H   Y   P   V   E   V   M   L   K   *
```

FIGURE 6

(A) pAS27

LOCUS PAS27.DNA 1584 bp mRNA PRI 06-MAR-1995
DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40 NLS(construct 1)
ACCESSION
NID
KEYWORDS DNase I.
SOURCE DNase I sequence is from assembled oligos (thus modified c/f MHDNASE1.dna)
ORGANISM Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.
TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
MEDLINE 91067672
BASE COUNT 354 a 474 c 446 g 310 t
ORIGIN

```
   1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
  61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GGAGCGGCGG GCTGAAGATC
 781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC
 841 TACATTGTCC AGATCCTGAG CCGCTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC
 901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT
 961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCGTG
1021 TACAGGCCTG ACCAGGTGTC TGCGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC
1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCTTCTC CCGGTTCACA
1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCCTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG
1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGGACGTC
1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC
1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA
1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGGTTG CAGGGATGCT GCTCCGAGGG
1441 GCCGTTGTTC CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC
1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCAGTGGAGG TGATGCTGAA GGGGGGCGGA
1561 CCCAAAAAGA AGCGCAAGGT TTGA
```

//

↳ NLS

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File : DAS27.DNA
Range : 1 - 1584 Mode : Normal
Codon Table : Universal

FIGURE 6(B)

```
                9                  18                  27                  36                  45                  54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63                  72                  81                  90                  99                 108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117                 126                 135                 144                 153                 162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171                 180                 189                 198                 207                 216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225                 234                 243                 252                 261                 270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279                 288                 297                 306                 315                 324
    AGA GAC ACA TCC ACA AAC ACA CCC TAC ATG GAG CTC AGC AGC CTC ACC TCT GAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333                 342                 351                 360                 369                 378
    GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387                 396                 405                 414                 423                 432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441                 450                 459                 468                 477                 486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495                 504                 513                 522                 531                 540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549                 558                 567                 576                 585                 594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA CCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G
```


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```
          603                 612                 621                 630                 639                 648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

          657                 666                 675                 684                 693                 702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

          711                 720                 729                 738                 747                 756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

          765                 774                 783                 792                 801                 810
GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   G   S   G   G   L   K   I   A   A   F   N   I   Q   T   F   G   E

          819                 828                 837                 846                 855                 864
ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   K   M   S   N   A   T   L   V   S   Y   I   V   Q   I   L   S   R

          873                 882                 891                 900                 909                 918
TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   D   I   A   L   V   Q   E   V   R   D   S   H   L   T   A   V   G

          927                 936                 945                 954                 963                 972
AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   L   L   D   N   L   N   Q   D   A   P   D   T   Y   H   Y   V   V

          981                 990                 999                1008                1017                1026
AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   E   P   L   G   R   N   S   Y   K   E   R   Y   L   F   V   Y   R

         1035                1044                1053                1062                1071                1080
CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   D   Q   V   S   A   V   D   S   Y   Y   Y   D   D   G   C   E   P

         1089                1098                1107                1116                1125                1134
TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   G   N   D   T   F   N   R   E   P   A   I   V   R   F   F   S   R

         1143                1152                1161                1170                1179                1188
TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT CCC CCC CCC CCC CAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   T   E   V   R   E   F   A   I   V   P   L   H   A   A   P   G   D

         1197                1206                1215                1224                1233                1242
```


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```
GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   V   A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q   E   K

       1251        1260        1269        1278        1287        1296
TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 W   G   L   E   D   V   M   L   M   G   D   F   N   A   G   C   S   Y

       1305        1314        1323        1332        1341        1350
GTG AGA CCC TCC CAG TGG TCA TCC ATC CCC CTC TCG ACA AGC CCC ACC TTC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   R   P   S   Q   W   S   S   I   R   L   W   T   S   P   T   F   Q

       1359        1368        1377        1386        1395        1404
TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 W   L   I   P   D   S   A   D   T   T   A   T   P   T   H   C   A   Y

       1413        1422        1431        1440        1449        1458
CAC ACG ATC GTG GTT GCA GGG ATG CTG CTC CCA CCC CCC GTT GTT CCC GAC TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   R   I   V   V   A   G   M   L   L   R   G   A   V   V   P   D   S

       1467        1476        1485        1494        1503        1512
GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   L   P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q

       1521        1530        1539        1548        1557        1566
GCC ATC AGT GAC CAC TAT CCA CTC CAC CTC ATC CTG AAG GGG GGC GGA CCC AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   I   S   D   H   Y   P   V   E   V   M   L   K   G   G   G   P   K

       1575        1584
AAG AAG CGC AAG GTT TGA 3'
--- --- --- --- --- ---
 K   K   R   K   V   *
```


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FIGURE 7

(A) pAS34

```
LOCUS       PAS34.DNA    2196 bp        2196 bp  2196 bp  DNA   14-AUG-1998
DEFINITION  HUMANISED HMFG1 heavy chain fused to human DNAse construct 34
DEFINITION  Clone 16.4.2 (same as hcdnase1.dna template file)
REFERENCE
  AUTHORS   VERHOEYEN ET AL
  TITLE     CONSTRUCTION OF RESHAPED HMFG1 etc
  JOURNAL   IMMUNOL. (1993):78, 364-370
COMMENT     Human DNAse sequence is modified as a result of oligo assembly
(mhdnase.dna)
COMMENT     The fusion was made using overlapping oligos AS79 and AS80
FEATURES    AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES    Residue 963 is G > T leading to silent mutation in all clones
  SITES     Note
BASE COUNT      501 a    677 c    607 g    411 t
ORIGIN          ?
```

```
   1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
  61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
 551 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGGGTAA AGGGAGCGGC GGGCTGAAGA TCGCAGCCTT CAACATCCAG
1441 ACATTTGGGG AGACCAAGAT GTCCAATGCC ACCCTCGTCA GCTACATTGT GCAGATCCTG
1501 AGCCGCTACG ACATCGCCCT GGTCCAGGAG GTCAGAGACA GCCACCTGAC TGCCGTGGGG
1561 AAGCTGCTGG ACAACCTCAA TCAGGACGCA CCAGACACCT ATCACTACGT GGTCAGTGAG
1621 CCACTGGGAC GGAACAGCTA TAAGGAGCGC TACCTGTTCG TGTACAGGCC TGACCAGGTG
1681 TCTGCGGTGG ACAGCTACTA CTACGATGAT GGCTGCGAGC CCTGCGGGAA CGACACCTTC
1741 AACCGAGAGC CAGCCATTGT CAGGTTCTTC TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC
1801 ATTGTTCCCC TGCATGCGGC CCCGGGGGAC GCAGTAGCCG AGATCGACGC TCTCTATGAC
1861 GTCTACCTGG ATGTCCAAGA GAAATGGGGC TTGGAGGACG TCATGTTGAT GGGCGACTTC
1921 AATGCGGGCT GCAGCTATGT GAGACCCTCC CAGTGGTCAT CCATCCGCCT GTGGACAAGC
1981 CCCACCTTCC AGTGGCTGAT CCCCGACAGC GCTGACACCA CAGCTACACC CACGCACTGT
2041 GCCTATGACA GGATCGTGGT TGCAGGGATG CTGCTCCGAG GGGCCGTTGT TCCCGACTCG
2101 GCTCTTCCCT TTAACTTCCA GGCTGCCTAT GGCCTGAGTG ACCAACTGGC CCAAGCCATC
2161 AGTGACCACT ATCCAGTGGA GGTGATGCTG AAGTGA
//
```

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File : PAS34.DNA
Range : 1 - 2196 Mode : Normal
Codon Table : Universal

FIGURE 7(B)

```
                9                  18                  27                  36                  45                  54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63                  72                  81                  90                  99                 108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117                 126                 135                 144                 153                 162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171                 180                 189                 198                 207                 216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225                 234                 243                 252                 261                 270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279                 288                 297                 306                 315                 324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333                 342                 351                 360                 369                 378
    CAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387                 396                 405                 414                 423                 432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441                 450                 459                 468                 477                 486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495                 504                 513                 522                 531                 540
    CCC TCC CTC CTC AAC GAC TAC TTC CCC GAA CCC CTC ACG GTG TCC TCC AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549                 558                 567                 576                 585                 594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G

              603                 612                 621                 630                 639                 648
    CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

              657                 666                 675                 684                 693                 702
```


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```
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

        711         720         729         738         747         756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

        765         774         783         792         801         810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   D   T

        819         828         837         846         855         864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H

        873         882         891         900         909         918
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N

        927         936         945         954         963         972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S

        981         990         999        1008        1017        1026
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K

       1035        1044        1053        1062        1071        1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A   K

       1089        1098        1107        1116        1125        1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D   E   L

       1143        1152        1161        1170        1179        1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D

       1197        1206        1215        1224        1233        1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T

       1251        1260        1269        1278        1287        1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V

       1305        1314        1323        1332        1341        1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E
```

1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA GGG					
A L H N H Y T Q K S L S L S P G K G					

1413	1422	1431	1440	1449	1458
AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG					
S G G L K I A A F N I Q T F G E T K					

1467	1476	1485	1494	1503	1512
ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC					
M S N A T L V S Y I V Q I L S R Y D					

1521	1530	1539	1548	1557	1566
ATC CCC CTC CTC CAG CAG CTC AGA CAC ACC CAC CTG ACT GCC GTG GGG AAG CTG					
I A L V Q E V R D S H L T A V G K L					

1575	1584	1593	1602	1611	1620
CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG					
L D N L N Q D A P D T Y H Y V V S E					

1629	1638	1647	1656	1665	1674
CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC					
P L G R N S Y K E R Y L F V Y R P D					

1683	1692	1701	1710	1719	1728
CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG					
Q V S A V D S Y Y Y D D G C E P C G					

1737	1746	1755	1764	1773	1782
AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA					
N D T F N R E P A I V R F F S R F T					

1791	1800	1809	1818	1827	1836
GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA					
E V R E F A I V P L H A A P G D A V					

1845	1854	1863	1872	1881	1890
GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC					
A E I D A L Y D V Y L D V Q E K W G					

1899	1908	1917	1926	1935	1944
TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA					
L E D V M L M G D F N A G C S Y V R					

1953	1962	1971	1980	1989	1996
CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG					
P S Q W S S I R L W T S P T F Q W L					

2007	2016	2025	2034	2043	2052
ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG					
I P D S A D T T A T P T H C A Y D R					

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```
      2061       2070       2079       2088       2097       2106
ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   V   V   A   G   M   L   L   R   G   A   V   V   P   D   S   A   L

      2115       2124       2133       2142       2151       2160
CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTC GCC CAA GCC ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q   A   I

      2169       2178       2187       2196
AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'
--- --- --- --- --- --- --- --- --- --- --- ---
 S   D   H   Y   P   V   E   V   M   L   K   *
```

FIGURE 8

(A) pAS35

```
LOCUS       PAS35.DNA    2193 bp   2193 bp    DNA                14-AUG-1998
DEFINITION  HUMANISED HMFG1 heavy chain fused to human DNAse construct 35
DEFINITION  Clone 17.12.1 with silent K to K mutation (1398 A > G)
REFERENCE
  AUTHORS   VERHOEYEN ET AL
  TITLE     CONSTRUCTION OF RESHAPED HMFG1 etc
  JOURNAL   IMMUNOL. (1993):78, 364-370
COMMENT     Human DNAse sequence is modified as a result of oligo assembly
(mhdnase.dna)
COMMENT     The fusion was made using overlapping oligos AS81 and AS82
FEATURES    AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES    Residue 963 is G > T leading to silent mutation in all clones
FEATURES    In 17.12.1 residue 1398 is A > G (silent K to K mutation)
  SITES     Note
BASE COUNT      500 a    677 c    606 g    410 t
ORIGIN          ?
```

```
   1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
  61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGAAgGG GAGCGGCGGG CTGAAGATCG CAGCCTTCAA CATCCAGACA
1441 TTTGGGGAGA CCAAGATGTC CAATGCCACC CTCGTCAGCT ACATTGTGCA GATCCTGAGC
1501 CGCTACGACA TCGCCCTGGT CCAGGAGGTC AGAGACAGCC ACCTGACTGC CGTGGGGAAG
1561 CTGCTGGACA ACCTCAATCA GGACGCACCA GACACCTATC ACTACGTGGT CAGTGAGCCA
1621 CTGGGACGGA ACAGCTATAA GGAGCGCTAC CTGTTCGTGT ACAGGCCTGA CCAGGTGTCT
1681 GCGGTGGACA GCTACTACTA CGATGATGGC TGCGAGCCCT GCGGGAACGA CACCTTCAAC
1741 CGAGAGCCAG CCATTGTCAG GTTCTTCTCC CGGTTCACAG AGGTCAGGGA GTTTGCCATT
1801 GTTCCCCTGC ATGCGGCCCC GGGGGACGCA GTAGCCGAGA TCGACGCTCT CTATGACGTC
1861 TACCTGGATG TCCAAGAGAA ATGGGGCTTG GAGGACGTCA TGTTGATGGG CGACTTCAAT
1921 GCGGGCTGCA GCTATGTGAG ACCCTCCCAG TGGTCATCCA TCCGCCTGTG GACAAGCCCC
1981 ACCTTCCAGT GGCTGATCCC CGACAGCGCT GACACCACAG CTACACCCAC GCACTGTGCC
2041 TATGACAGGA TCGTGGTTGC AGGGATGCTG CTCCGAGGGG CCGTTGTTCC CGACTCGGCT
2101 CTTCCCTTTA ACTTCCAGGC TGCCTATGGC CTGAGTGACC AACTGGCCCA AGCCATCAGT
2161 GACCACTATC CAGTGGAGGT GATGCTGAAG TGA
//
```

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File : PAS35.DNA
Range : 1 - 2193 Mode : Normal
Codon Table : Universal

FIGURE 8(B)

```
                9                  18                  27                  36                  45                  54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63                  72                  81                  90                  99                 108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117                 126                 135                 144                 153                 162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171                 180                 189                 198                 207                 216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225                 234                 243                 252                 261                 270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279                 288                 297                 306                 315                 324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333                 342                 351                 360                 369                 378
    GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387                 396                 405                 414                 423                 432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441                 450                 459                 468                 477                 486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495                 504                 513                 522                 531                 540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549                 558                 567                 576                 585                 594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G

              603                 612                 621                 630                 639                 648
    CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

              657                 666                 675                 684                 693                 702
```


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```
ACC TAC ATC TCC AAC GTC AAT CAC AAG CCC ACC AAC ACC AAC GTC CAC AAC AAA
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 T    Y    I    C    N    V    N    H    K    P    S    N    T    K    V    D    K    K

          711            720            729            738            747            756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 V    E    P    K    S    C    D    K    T    H    T    C    P    P    C    P    A    P

          765            774            783            792            801            810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 E    L    L    G    G    P    S    V    F    L    F    P    P    K    P    K    D    T

          819            828            837            846            855            864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTC AGC CAC
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 L    M    I    S    R    T    P    E    V    T    C    V    V    V    D    V    S    H

          873            882            891            900            909            918
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 E    D    P    E    V    K    F    N    W    Y    V    D    G    V    E    V    H    N

          927            936            945            954            963            972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 A    K    T    K    P    R    E    E    Q    Y    N    S    T    Y    R    V    V    S

          981            990            999            1008           1017           1026
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 V    L    T    V    L    H    Q    D    W    L    N    G    K    E    Y    K    C    K

          1035           1044           1053           1062           1071           1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 V    S    N    K    A    L    P    A    P    I    E    K    T    I    S    K    A    K

          1089           1098           1107           1116           1125           1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 G    Q    P    R    E    P    Q    V    Y    T    L    P    P    S    R    D    E    L

          1143           1152           1161           1170           1179           1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 T    K    N    Q    V    S    L    T    C    L    V    K    G    F    Y    P    S    D

          1197           1206           1215           1224           1233           1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 I    A    V    E    W    E    S    N    G    Q    P    E    N    N    Y    K    T    T

          1251           1260           1269           1278           1287           1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 P    P    V    L    D    S    D    G    S    F    F    L    Y    S    K    L    T    V

          1305           1314           1323           1332           1341           1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---  ---
 D    K    S    R    W    Q    Q    G    N    V    F    S    C    S    V    M    H    E
```


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1359	1368	1377	1386	1395	1404
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG AAG GGG AGC					
A L H N H Y T Q K S L S L S P K G S					

1413	1422	1431	1440	1449	1458
GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG					
G G L K I A A F N I Q T F G E T K M					

1467	1476	1485	1494	1503	1512
TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC ATC					
S N A T L V S Y I V Q I L S R Y D I					

1521	1530	1539	1548	1557	1566
GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG CTG					
A L V Q E V R D S H L T A V G K L L					

1575	1584	1593	1602	1611	1620
CAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA					
D N L N Q D A P D T Y H Y V V S E P					

1629	1638	1647	1656	1665	1674
CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG					
L G R N S Y K E R Y L F V Y R P D Q					

1683	1692	1701	1710	1719	1728
GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG AAC					
V S A V D S Y Y Y D D G C E P C G N					

1737	1746	1755	1764	1773	1782
GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG					
D T F N R E P A I V R F F S R F T E					

1791	1800	1809	1818	1827	1836
GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC					
V R E F A I V P L H A A P G D A V A					

1845	1854	1863	1872	1881	1890
GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG					
E I D A L Y D V Y L D V Q E K W G L					

1899	1908	1917	1926	1935	1944
GAG GAC GTC ATC TTG ATG CCC CAC TTC AAT CCC GCC TGC ACC TAT GTC ACA CCC					
E D V M L M G D F N A G C S Y V R P					

1953	1962	1971	1980	1989	1998
TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC					
S Q W S S I R L W T S P T F Q W L I					

2007	2016	2025	2034	2043	2052
CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC					
P D S A D T T A T P T H C A Y D R I					

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```
          2061       2070       2079       2088       2097       2106
GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   V   A   G   M   L   L   R   G   A   V   V   P   D   S   A   L   P

          2115       2124       2133       2142       2151       2160
TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q   A   I   S

          2169       2178       2187
GAC CAC TAT CCA GTG GAC CTC ATC CTC AAG TCA 3'
--- --- --- --- --- --- --- --- --- --- ---
 D   H   Y   P   V   E   V   M   L   K   *
```


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FIGURE 9

(A) pAS36

```
LOCUS       PAS36.DNA    2190 bp  2190 bp    DNA                    14-AUG-1998
DEFINITION  HUMANISED HMFG1 heavy chain fused to human DNAse - construct 36
DEFINITION  Clone 18.24.1 with residue 1392 T > C
REFERENCE
  AUTHORS   VERHOEYEN ET AL
  TITLE     CONSTRUCTION OF RESHAPED HMFG1 etc
  JOURNAL   IMMUNOL. (1993):78, 364-370
COMMENT     Human DNAse sequence is modified as a result of oligo assembly
(mndnase.dna)
COMMENT     The fusion was made using overlapping oligos AS83 and AS84
FEATURES    AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES    Residue 963 is G > T leading to silent mutation in all clones
FEATURES    Residue 1392 T > C silent S to S mutation
  SITES     Note
BASE COUNT      498 a    678 c    605 g    409 t
ORIGIN          ?
```

```
   1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
  61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CcCCGGGGAG CGGCGGGCTG AAGATCGCAG CCTTCAACAT CCAGACATTT
1441 GGGGAGACCA AGATGTCCAA TGCCACCCTC GTCAGCTACA TTGTGCAGAT CCTGAGCCGC
1501 TACGACATCG CCCTGGTCCA GGAGGTCAGA GACAGCCACC TGACTGCCGT GGGGAAGCTG
1561 CTGGACAACC TCAATCAGGA CGCACCAGAC ACCTATCACT ACGTGGTCAG TGAGCCACTG
1621 GGACGGAACA GCTATAAGGA GCGCTACCTG TTCGTGTACA GGCCTGACCA GGTGTCTGCG
1681 GTGGACAGCT ACTACTACGA TGATGGCTGC GAGCCCTGCG GGAACGACAC CTTCAACCGA
1741 GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG TTCACAGAGG TCAGGGAGTT TGCCATTGTT
1801 CCCCTGCATG CGGCCCCGGG GGACGCAGTA GCCGAGATCG ACGCTCTCTA TGACGTCTAC
1861 CTGGATGTCC AAGAGAAATG GGGCTTGGAG GACGTCATGT TGATGGGCGA CTTCAATGCG
1921 GGCTGCAGCT ATGTGAGACC CTCCCAGTGG TCATCCATCC GCCTGTGGAC AAGCCCCACC
1981 TTCCAGTGGC TGATCCCCGA CAGCGCTGAC ACCACAGCTA CACCCACGCA CTGTGCCTAT
2041 GACAGGATCG TGGTTGCAGG GATGCTGCTC CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT
2101 CCCTTTAACT TCCAGGCTGC CTATGGCCTG AGTGACCAAC TGGCCCAAGC CATCAGTGAC
2161 CACTATCCAG TGGAGGTGAT GCTGAAGTGA
```

//

File : PAS36.DNA
Range : 1 - 2190 Mode : Normal
Codon Table : Universal

FIGURE 9 (B)

```
                9                  18                  27                  36                  45                  54
5'  ATC CCA TCC ACC TCT ATC ATC CTC TTC TTC CTA GCA ACA GCT ACA CCT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63                  72                  81                  90                  99                 108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117                 126                 135                 144                 153                 162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171                 180                 189                 198                 207                 216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225                 234                 243                 252                 261                 270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279                 288                 297                 306                 315                 324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333                 342                 351                 360                 369                 378
    GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387                 396                 405                 414                 423                 432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441                 450                 459                 468                 477                 486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495                 504                 513                 522                 531                 540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549                 558                 567                 576                 585                 594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G

              603                 612                 621                 630                 639                 648
    CTC TAC TCC CTC AGC AGC GTG GTG ACC GTC CCC TCC AGC AGC TTG GGC ACC CAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

              657                 666                 675                 684                 693                 702
```


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```
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

          711         720         729         738         747         756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
 V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

          765         774         783         792         801         810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
 E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   D   T

          819         828         837         846         855         864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
 L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H

          873         882         891         900         909         918
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
 E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N

          927         936         945         954         963         972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
 A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S

        981         990         999        1008        1017        1026
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
 V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K

       1035        1044        1053        1062        1071        1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
 V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A   K

       1089        1098        1107        1116        1125        1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
 G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D   E   L

       1143        1152        1161        1170        1179        1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
 T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D

       1197        1206        1215        1224        1233        1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
 I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T

          1251        1260        1269        1278        1287        1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
 P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V

       1305        1314        1323        1332        1341        1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
 D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E
```


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```
      1359       1368       1377       1386       1395       1404
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCC CCG GGG AGC GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   L   H   N   H   Y   T   Q   K   S   L   S   L   S   P   G   S   G

      1413       1422       1431       1440       1449       1458
GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   L   K   I   A   A   F   N   I   Q   T   F   G   E   T   K   M   S

      1467       1476       1485       1494       1503       1512
AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC ATC GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   A   T   L   V   S   Y   I   V   Q   I   L   S   R   Y   D   I   A

      1521       1530       1539       1548       1557       1566
CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG CTG GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   V   Q   E   V   R   D   S   H   L   T   A   V   G   K   L   L   D

      1575       1584       1593       1602       1611       1620
AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   L   N   Q   D   A   P   D   T   Y   H   Y   V   V   S   E   P   L

      1629       1638       1647       1656       1665       1674
GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   R   N   S   Y   K   E   R   Y   L   F   V   Y   R   P   D   Q   V

      1683       1692       1701       1710       1719       1728
TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG AAC GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   A   V   D   S   Y   Y   Y   D   D   G   C   E   P   C   G   N   D

      1737       1746       1755       1764       1773       1782
ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG GTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   F   N   R   E   P   A   I   V   R   F   F   S   R   F   T   E   V

      1791       1800       1809       1818       1827       1836
AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   E   F   A   I   V   P   L   H   A   A   P   G   D   A   V   A   E

      1845       1854       1863       1872       1881       1890
ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   D   A   L   Y   D   V   Y   L   D   V   Q   E   K   W   G   L   E

      1899       1908       1917       1926       1935       1944
GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA CCC TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   V   M   L   M   G   D   F   N   A   G   C   S   Y   V   R   P   S

      1953       1962       1971       1980       1989       1998
CAG TGG TCA TCC ATC CGC CTG TGG ACA ACC CCC ACC TTC CAG TGG CTG ATC CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   W   S   S   I   R   L   W   T   S   P   T   F   Q   W   L   I   P

      2007       2016       2025       2034       2043       2052
GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   S   A   D   T   T   A   T   P   T   H   C   A   Y   D   R   I   V
```

```
          2061        2070        2079        2088        2097        2106
GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   A   G   M   L   L   R   G   A   V   V   P   D   S   A   L   P   F

          2115        2124        2133        2142        2151        2160
AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q   A   I   S   D

          2169        2178        2187
CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'
--- --- --- --- --- --- --- --- --- ---
 H   Y   P   V   E   V   M   L   K   *
```


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FIGURE 10

(A) pAS37

```
LOCUS       PAS37.DNA    2226 bp        2196 bp  2196 bp DNA      14-AUG-1998
DEFINITION  HUMANISED HMFG1 heavy chain fused to human DNAse construct 37
DEFINITION  Clone 16.4.2 (same as hcdnase1.dna template file) plus NLS
REFERENCE
  AUTHORS   VERHOEYEN ET AL
  TITLE     CONSTRUCTION OF RESHAPED HMFG1 etc
  JOURNAL   IMMUNOL. (1993):78, 364-370
COMMENT     Human DNAse sequence is modified as a result of oligo assembly
(mhdnase.dna)
COMMENT     The fusion was made using overlapping oligos AS79 and AS80
FEATURES    AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES    Residue 963 is G > T leading to silent mutation in all clones
  SITES     Note
BASE COUNT      511 a    683 c    619 g    413 t
ORIGIN           ?
```

```
   1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
  61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGGGTAA AGGGAGCGGC GGGCTGAAGA TCGCAGCCTT CAACATCCAG
1441 ACATTTGGGG AGACCAAGAT GTCCAATGCC ACCCTCGTCA GCTACATTGT GCAGATCCTG
1501 AGCCGCTACG ACATCGCCCT GGTCCAGGAG GTCAGAGACA GCCACCTGAC TGCCGTGGGG
1561 AAGCTGCTGG ACAACCTCAA TCAGGACGCA CCAGACACCT ATCACTACGT GGTCAGTGAG
1621 CCACTGGGAC GGAACAGCTA TAAGGAGCGC TACCTGTTCG TGTACAGGCC TGACCAGGTG
1681 TCTGCGGTGG ACAGCTACTA CTACGATGAT GGCTGCGAGC CCTGCGGGAA CGACACCTTC
1741 AACCGAGAGC CAGCCATTGT CAGGTTCTTC TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC
1801 ATTGTTCCCC TGCATGCGGC CCCGGGGGAC GCAGTAGCCG AGATCGACGC TCTCTATGAC
1861 GTCTACCTGG ATGTCCAAGA GAAATGGGGC TTGGAGGACG TCATGTTGAT GGGCGACTTC
1921 AATGCGGGCT GCAGCTATGT GAGACCCTCC CAGTGGTCAT CCATCCGCCT GTGGACAAGC
1981 CCCACCTTCC AGTGGCTGAT CCCCGACAGC GCTGACACCA CAGCTACACC CACGCACTGT
2041 GCCTATGACA GGATCGTGGT TGCAGGGATG CTGCTCCGAG GGGCCGTTGT TCCCGACTCG
2101 GCTCTTCCCT TTAACTTCCA GGCTGCCTAT GGCCTGAGTG ACCAACTGGC CCAAGCCATC
2161 AGTGACCACT ATCCAGTGGA GGTGATGCTG AAGGGGGGCG GACCCAAAAA GAAGCGCAAG
2221 GTTTGA
```

NLS

//

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File : PAS37.DNA
Range : 1 - 2226 Mode : Normal
Codon Table : Universal

FIGURE 10 (B)

```
                9                  18                  27                  36                  45                  54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63                  72                  81                  90                  99                 108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117                 126                 135                 144                 153                 162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171                 180                 189                 198                 207                 216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225                 234                 243                 252                 261                 270
    GGA AGT AAT AAT TCT ACA TAC AAT CAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279                 288                 297                 306                 315                 324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333                 342                 351                 360                 369                 378
    GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387                 396                 405                 414                 423                 432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441                 450                 459                 468                 477                 486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495                 504                 513                 522                 531                 540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549                 558                 567                 576                 585                 594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G

              603                 612                 621                 630                 639                 648
    CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

              657                 666                 675                 684                 693                 702
```


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```
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

          711         720         729         738         747         756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

          765         774         783         792         801         810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   D   T

          819         828         837         846         855         864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H

          873         882         891         900         909         918
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N

          927         936         945         954         963         972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S

        981         990         999        1008        1017        1026
CTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K

       1035        1044        1053        1062        1071        1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A   K

       1089        1098        1107        1116        1125        1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D   E   L

       1143        1152        1161        1170        1179        1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D

       1197        1206        1215        1224        1233        1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T

       1251        1260        1269        1278        1287        1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V

       1305        1314        1323        1332        1341        1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---
 D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E
```


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```
      1359       1368       1377       1386       1395       1404
GCT CTG CAC AAC CAC TAC ACC CAG AAG AGC CTC TCC CTC TCT CCC GGT AAA CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   L   H   N   H   Y   T   Q   K   S   L   S   L   S   P   G   K   G

      1413       1422       1431       1440       1449       1458
AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   G   G   L   K   I   A   A   F   N   I   Q   T   F   G   E   T   K

      1467       1476       1485       1494       1503       1512
ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   S   N   A   T   L   V   S   Y   I   V   Q   I   L   S   R   Y   D

      1521       1530       1539       1548       1557       1566
ATC CCC CTG CTC CAC GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GCC AAG CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   A   L   V   Q   E   V   R   D   S   H   L   T   A   V   G   K   L

      1575       1584       1593       1602       1611       1620
CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   D   N   L   N   Q   D   A   P   D   T   Y   H   Y   V   V   S   E

      1629       1638       1647       1656       1665       1674
CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   L   G   R   N   S   Y   K   E   R   Y   L   F   V   Y   R   P   D

      1683       1692       1701       1710       1719       1728
CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC CAT CAT CCC TGC GAG CCC TGC GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   V   S   A   V   D   S   Y   Y   Y   D   D   G   C   E   P   C   G

      1737       1746       1755       1764       1773       1782
AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   D   T   F   N   R   E   P   A   I   V   R   F   F   S   R   F   T

      1791       1800       1809       1818       1827       1836
GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   V   R   E   F   A   I   V   P   L   H   A   A   P   G   D   A   V

      1845       1854       1863       1872       1881       1890
GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q   E   K   W   G

      1899       1908       1917       1926       1935       1944
TTG GAC GAC CTC ATC TTC ATC GCC GAC TTC AAT GCG GGC TCC ACC TAT GTG AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   E   D   V   M   L   M   G   D   F   N   A   G   C   S   Y   V   R

      1953       1962       1971       1980       1989       1998
CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   S   Q   W   S   S   I   R   L   W   T   S   P   T   F   Q   W   L

      2007       2016       2025       2034       2043       2052
ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   P   D   S   A   D   T   T   A   T   P   T   H   C   A   Y   D   R
```


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```
      2061       2070       2079       2088       2097       2106
ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   V   V   A   G   M   L   L   R   G   A   V   V   P   D   S   A   L

      2115       2124       2133       2142       2151       2160
CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q   A   I

      2169       2178       2187       2196       2205       2214
AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   D   H   Y   P   V   E   V   M   L   K   G   G   G   P   K   K   K

      2223
CGC AAG GTT TGA 3'
--- --- --- ---
 R   K   V   *
```


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FIGURE 11

(A) pAS38

```
LOCUS       PAS38.DNA    2223 bp   2193 bp    DNA              14-AUG-1998
DEFINITION  HUMANISED HMFG1 heavy chain fused to human DNAse construct 38
DEFINITION  Clone 17.12.1 with silent K to K mutation (1398 A > G)+NLS
REFERENCE
  AUTHORS   VERHOEYEN ET AL
  TITLE     CONSTRUCTION OF RESHAPED HMFG1 etc
  JOURNAL   IMMUNOL. (1993):78, 364-370
COMMENT     Human DNAse sequence is modified as a result of oligo assembly
(mhdnase.dna)
COMMENT     The fusion was made using overlapping oligos AS81 and AS82
FEATURES    AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES    Residue 963 is G > T leading to silent mutation in all clones
FEATURES    In 17.12.1 residue 1398 is A > G (silent K to K mutation)
  SITES     Note
BASE COUNT      510 a    683 c    618 g    412 t
ORIGIN          ?
```

```
   1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
  61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGAAgGG GAGCGGCGGG CTGAAGATCG CAGCCTTCAA CATCCAGACA
1441 TTTGGGGAGA CCAAGATGTC CAATGCCACC CTCGTCAGCT ACATTGTGCA GATCCTGAGC
1501 CGCTACGACA TCGCCCTGGT CCAGGAGGTC AGAGACAGCC ACCTGACTGC CGTGGGGAAG
1561 CTGCTGGACA ACCTCAATCA GGACGCACCA GACACCTATC ACTACGTGGT CAGTGAGCCA
1621 CTGGGACGGA ACAGCTATAA GGAGCGCTAC CTGTTCGTGT ACAGGCCTGA CCAGGTGTCT
1681 GCGGTGGACA GCTACTACTA CGATGATGGC TGCGAGCCCT GCGGGAACGA CACCTTCAAC
1741 CGAGAGCCAG CCATTGTCAG GTTCTTCTCC CGGTTCACAG AGGTCAGGGA GTTTGCCATT
1801 GTTCCCCTGC ATGCGGCCCC GGGGGACGCA GTAGCCGAGA TCGACGCTCT CTATGACGTC
1861 TACCTGGATG TCCAAGAGAA ATGGGGCTTG GAGGACGTCA TGTTGATGGG CGACTTCAAT
1921 GCGGGCTGCA GCTATGTGAG ACCCTCCCAG TGGTCATCCA TCCGCCTGTG GACAAGCCCC
1981 ACCTTCCAGT GGCTGATCCC CGACAGCGCT GACACCACAG CTACACCCAC GCACTGTGCC
2041 TATGACAGGA TCGTGGTTGC AGGGATGCTG CTCCGAGGGG CCGTTGTTCC CGACTCGGCT
2101 CTTCCCTTTA ACTTCCAGGC TGCCTATGGC CTGAGTGACC AACTGGCCCA AGCCATCAGT
2161 GACCACTATC CAGTGGAGGT GATGCTGAAG GGGGGCGGAC CCAAAAAGAA GCGCAAGGTT
2221 TGA
//
```

NLS

File : PAS38.DNA
Range : 1 - 2223 Mode : Normal
Codon Table : Universal

FIGURE 11 (B)

```
                9                  18                  27                  36                  45                  54
5'  ATG GCA TGG ACC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63                  72                  81                  90                  99                 108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTC AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117                 126                 135                 144                 153                 162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171                 180                 189                 198                 207                 216
    TCC GTG CGC CAG CCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225                 234                 243                 252                 261                 270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAC CCC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279                 288                 297                 306                 315                 324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333                 342                 351                 360                 369                 378
    GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387                 396                 405                 414                 423                 432
    TGG GGC CAA GGG ACT CTC GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441                 450                 459                 468                 477                 486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495                 504                 513                 522                 531                 540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549                 558                 567                 576                 585                 594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G

              603                 612                 621                 630                 639                 648
    CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

              657                 666                 675                 684                 693                 702
```


B7
82

```
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
---------------------------------------------------------------------
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

          711             720             729             738             747             756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
---------------------------------------------------------------------
 V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

          765             774             783             792             801             810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
---------------------------------------------------------------------
 E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   D   T

          819             828             837             846             855             864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
---------------------------------------------------------------------
 L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H

          873             882             891             900             909             918
CAA CAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
---------------------------------------------------------------------
 E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N

          927             936             945             954             963             972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
---------------------------------------------------------------------
 A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S

          981             990             999            1008            1017            1026
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG
---------------------------------------------------------------------
 V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K

         1035            1044            1053            1062            1071            1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
---------------------------------------------------------------------
 V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A   K

         1089            1098            1107            1116            1125            1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
---------------------------------------------------------------------
 G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D   E   L

         1143            1152            1161            1170            1179            1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
---------------------------------------------------------------------
 T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D

         1197            1206            1215            1224            1233            1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
---------------------------------------------------------------------
 I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T

         1251            1260            1269            1278            1287            1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
---------------------------------------------------------------------
 P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V

         1305            1314            1323            1332            1341            1350
CAC AAC ACC ACC TCC CAC CAG GGG AAC GTC TTC TCA TCC TCC GTG ATG CAT GAG
---------------------------------------------------------------------
 D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E
```


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1359	1368	1377	1386	1395	1404
CCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG AAG GGG AGC					
A L H N H Y T Q K S L S L S P K G S					

1413	1422	1431	1440	1449	1458
GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG					
G G L K I A A F N I Q T F G E T K M					

1467	1476	1485	1494	1503	1512
TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG ACC CGC TAC GAC ATC					
S N A T L V S Y I V Q I L S R Y D I					

1521	1530	1539	1548	1557	1566
GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG CTG					
A L V Q E V R D S H L T A V G K L L					

1575	1584	1593	1602	1611	1620
GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA					
D N L N Q D A P D T Y H Y V V S E P					

1629	1638	1647	1656	1665	1674
CTG GGA CGG AAC AGC TAT AAC CAC CCC TAC CTG TTC GTG TAC AGG CCT GAC CAG					
L G R N S Y K E R Y L F V Y R P D Q					

1683	1692	1701	1710	1719	1728
GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG AAC					
V S A V D S Y Y Y D D G C E P C G N					

1737	1746	1755	1764	1773	1782
GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG					
D T F N R E P A I V R F F S R F T E					

1791	1800	1809	1818	1827	1836
GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC					
V R E F A I V P L H A A P G D A V A					

1845	1854	1863	1872	1881	1890
GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG					
E I D A L Y D V Y L D V Q E K W G L					

1899	1908	1917	1926	1935	1944
GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA CCC					
E D V M L M G D F N A G C S Y V R P					

1953	1962	1971	1980	1989	1998
TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC					
S Q W S S I R L W T S P T F Q W L I					

2007	2016	2025	2034	2043	2052
CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC					
P D S A D T T A T P T H C A Y D R I					

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```
      2061        2070        2079        2088        2097        2106
GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   V   A   G   M   L   L   R   G   A   V   V   P   D   S   A   L   P

      2115        2124        2133        2142        2151        2160
TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q   A   I   S

      2169        2178        2187        2196        2205        2214
GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   H   Y   P   V   E   V   M   L   K   G   G   G   P   K   K   K   R

      2223
AAG GTT TGA 3'
--- --- ---
 K   V   *
```


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FIGURE 12

(4) pAS39

```
LOCUS       PAS39.DNA     2220 bp  2190 bp     DNA                    14-AUG-1998
DEFINITION  HUMANISED HMFG1 heavy chain fused to human DNAse - construct 39
DEFINITION  Clone 18.24.1 with residue 1392 T > C +NLS
REFERENCE
  AUTHORS   VERHOEYEN ET AL
  TITLE     CONSTRUCTION OF RESHAPED HMFG1 etc
  JOURNAL   IMMUNOL. (1993):78, 364-370
COMMENT     Human DNAse sequence is modified as a result of oligo assembly
(mhdnase.dna)
COMMENT     The fusion was made using overlapping oligos AS83 and AS84
FEATURES    AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES    Residue 963 is G > T leading to silent mutation in all clones
FEATURES    Residue 1392 T > C silent S to S mutation
  SITES     Note
BASE COUNT      508 a    684 c    617 g    411 t
ORIGIN          ?

        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
      781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
      841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
      901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
      961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
     1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
     1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
     1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
     1201 TGGGAGAGCA ATGGGCAGCC GCAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
     1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
     1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
     1381 CTCTCCCTGT CCCCGGGGAG CGGCGGGCTG AAGATCGCAG CCTTCAACAT CCAGACATTT
     1441 GGGGAGACCA AGATGTCCAA TGCCACCCTC GTCAGCTACA TTGTGCAGAT CCTGAGCCGC
     1501 TACGACATCG CCCTGGTCCA GGAGGTCAGA GACAGCCACC TGACTGCCGT GGGGAAGCTG
     1561 CTGGACAACC TCAATCAGGA CGCACCAGAC ACCTATCACT ACGTGGTCAG TGAGCCACTG
     1621 GGACGGAACA GCTATAAGGA GCGCTACCTG TTCGTGTACA GGCCTGACCA GGTGTCTGCG
     1681 GTGGACAGCT ACTACTACGA TGATGGCTGC GAGCCCTGCG GGAACGACAC CTTCAACCGA
     1741 GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG TTCACAGAGG TCAGGGAGTT TGCCATTGTT
     1801 CCCCTGCATG CGGCCCCGGG GGACGCAGTA GCCGAGATCG ACGCTCTCTA TGACGTCTAC
     1861 CTGGATGTCC AAGAGAAATG GGGCTTGGAG GACGTCATGT TGATGGGCGA CTTCAATGCG
     1921 GGCTGCAGCT ATGTGAGACC CTCCCAGTGG TCATCCATCC GCCTGTGGAC AAGCCCCACC
     1981 TTCCAGTGGC TGATCCCCGA CAGCGCTGAC ACCACAGCTA CACCCACGCA CTGTGCCTAT
     2041 GACAGGATCG TGGTTGCAGG GATGCTGCTC CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT
     2101 CCCTTTAACT TCCAGGCTGC CTATGGCCTG AGTGACCAAC TGGCCCAAGC CATCAGTGAC
     2161 CACTATCCAG TGGAGGTGAT GCTGAAGGGG GGCGGACCCA AAAAGAAGCG CAAGGTTTGA
//
```

9H/87

File : PAS39.DNA
Range : 1 - 2220 Mode : Normal
Codon Table : Universal

FIGURE 12(B)

```
                9                  18                  27                  36                  45                  54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63                  72                  81                  90                  99                 108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117                 126                 135                 144                 153                 162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171                 180                 189                 198                 207                 216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225                 234                 243                 252                 261                 270
    GGA AGT AAT AAT TCT AGA TAC AAT GAC AAG TTC AAG GGC CCA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279                 288                 297                 306                 315                 324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333                 342                 351                 360                 369                 378
    GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387                 396                 405                 414                 423                 432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441                 450                 459                 468                 477                 486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495                 504                 513                 522                 531                 540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549                 558                 567                 576                 585                 594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G

              603                 612                 621                 630                 639                 648
    CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

              657                 666                 675                 684                 693                 702
```


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```
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG CAC AAC AAA
---------------------------------------------------------------------
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

          711             720             729             738             747             756
GTT GAC CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
---------------------------------------------------------------------
 V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

          765             774             783             792             801             810
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
---------------------------------------------------------------------
 E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   D   T

          819             828             837             846             855             864
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTC ACC CAC
---------------------------------------------------------------------
 L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H

          873             882             891             900             909             918
GAA CAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
---------------------------------------------------------------------
 E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N

      927             936             945             954             963             972
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
---------------------------------------------------------------------
 A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S

      981             990             999            1008            1017            1026
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAC TCC AAG
---------------------------------------------------------------------
 V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K

     1035            1044            1053            1062            1071            1080
GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
---------------------------------------------------------------------
 V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A   K

     1089            1098            1107            1116            1125            1134
GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
---------------------------------------------------------------------
 G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D   E   L

     1143            1152            1161            1170            1179            1188
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
---------------------------------------------------------------------
 T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D

     1197            1206            1215            1224            1233            1242
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
---------------------------------------------------------------------
 I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T

     1251            1260            1269            1278            1287            1296
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
---------------------------------------------------------------------
 P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V

     1305            1314            1323            1332            1341            1350
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
---------------------------------------------------------------------
 D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E
```


43
154

```
      1359       1368       1377       1386       1395       1404
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCC CCC GGG AGC GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   L   H   N   H   Y   T   Q   K   S   L   S   L   S   P   G   S   G

      1413       1422       1431       1440       1449       1458
GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   L   K   I   A   A   F   N   I   Q   T   F   G   E   T   K   M   S

      1467       1476       1485       1494       1503       1512
AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC ATC GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   A   T   L   V   S   Y   I   V   Q   I   L   S   R   Y   D   I   A

      1521       1530       1539       1548       1557       1566
CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG CCC AAC CTG CTG GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   V   Q   E   V   R   D   S   H   L   T   A   V   G   K   L   L   D

      1575       1584       1593       1602       1611       1620
AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   L   N   Q   D   A   P   D   T   Y   H   Y   V   V   S   E   P   L

      1629       1638       1647       1656       1665       1674
GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   R   N   S   Y   K   E   R   Y   L   F   V   Y   R   P   D   Q   V

      1683       1692       1701       1710       1719       1728
TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT CCC TGC GAG CCC TGC CCC AAC GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   A   V   D   S   Y   Y   Y   D   D   G   C   E   P   C   G   N   D

      1737       1746       1755       1764       1773       1782
ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG GTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   F   N   R   E   P   A   I   V   R   F   F   S   R   F   T   E   V

      1791       1800       1809       1818       1827       1836
AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   E   F   A   I   V   P   L   H   A   A   P   G   D   A   V   A   E

      1845       1854       1863       1872       1881       1890
ATC CAC CCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   D   A   L   Y   D   V   Y   L   D   V   Q   E   K   W   G   L   E

      1899       1908       1917       1926       1935       1944
GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA CCC TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   V   M   L   M   G   D   F   N   A   G   C   S   Y   V   R   P   S

      1953       1962       1971       1980       1989       1998
CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   W   S   S   I   R   L   W   T   S   P   T   F   Q   W   L   I   P

      2007       2016       2025       2034       2043       2052
GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   S   A   D   T   T   A   T   P   T   H   C   A   Y   D   R   I   V
```


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```
        2061        2070        2079        2088        2097        2106
GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   A   G   M   L   L   R   G   A   V   V   P   D   S   A   L   P   F

        2115        2124        2133        2142        2151        2160
AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q   A   I   S   D

        2169        2178        2187        2196        2205        2214
CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG CGC AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   Y   P   V   E   V   M   L   K   G   G   G   P   K   K   K   R   K

GTT TGA 3'
--- ---
 V   *
```


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FIGURE 13

(A) pAS101

LOCUS PAS101.DNA 1548 bp mRNA PRI 06-MAR-1995
DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS101)
ACCESSION
NID
KEYWORDS DNase I.
SOURCE DNase I sequence is from assembled oligos (thus modified c/f MHDNASE1.dna)
ORGANISM Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.
TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
MEDLINE 91067672
BASE COUNT 343 a 467 c 430 g 308 t
ORIGIN

```
   1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
  61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GCGGGGCTGAA GATCGCAGCC
 781 TTCAACATCC AGACATTTGG GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT
 841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTCAGAGA CAGCCACCTG
 901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC
 961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG
1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG
1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC
1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC
1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG
1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGGTC ATCCATCCGC
1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA
1381 CCCACGCACT GTGCCTATGA CAGGATCGTG GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT
1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG
1501 GCCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGTGA
```

11

File : PAS101.DNA
Range : 1 - 1548 Mode : Normal
Codon Table : Universal

FIGURE 13(B)

```
                9          18          27          36          45          54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTC GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   C   V   H

               63          72          81          90          99         108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117         126         135         144         153         162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171         180         189         198         207         216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225         234         243         252         261         270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279         288         297         306         315         324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG ACC TCT CAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333         342         351         360         369         378
    GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387         396         405         414         423         432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441         450         459         468         477         486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495         504         513         522         531         540
    CCC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549         558         567         576         585         594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G
```

```
          603        612        621        630        639        648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

          657        666        675        684        693        702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

          711        720        729        738        747        756
CTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

          765        774        783        792        801        810
GAA GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   G   G   L   K   I   A   A   F   N   I   Q   T   F   G   E   T   K

          819        828        837        846        855        864
ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   S   N   A   T   L   V   S   Y   I   V   Q   I   L   S   R   Y   D

          873        882        891        900        909        918
ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   A   L   V   Q   E   V   R   D   S   H   L   T   A   V   G   K   L

          927        936        945        954        963        972
CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   D   N   L   N   Q   D   A   P   D   T   Y   H   Y   V   V   S   E

          981        990        999       1008       1017       1026
CCA CTC CCA CCC AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   L   G   R   N   S   Y   K   E   R   Y   L   F   V   Y   R   P   D

         1035       1044       1053       1062       1071       1080
CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   V   S   A   V   D   S   Y   Y   Y   D   D   G   C   E   P   C   G

         1089       1098       1107       1116       1125       1134
AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   D   T   F   N   R   E   P   A   I   V   R   F   F   S   R   F   T

         1143       1152       1161       1170       1179       1188
GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   V   R   E   F   A   I   V   P   L   H   A   A   P   G   D   A   V

         1197       1206       1215       1224       1233       1242
```


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```
GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q   E   K   W   G

      1251        1260        1269        1278        1287        1296
TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   E   D   V   M   L   M   G   D   F   N   A   G   C   S   Y   V   R

      1305        1314        1323        1332        1341        1350
CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   S   Q   W   S   S   I   R   L   W   T   S   P   T   F   Q   W   L

      1359        1368        1377        1386        1395        1404
ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   P   D   S   A   D   T   T   A   T   P   T   H   C   A   Y   D   R

      1413        1422        1431        1440        1449        1458
ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCC CCT CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   V   V   A   G   M   L   L   R   G   A   V   V   P   D   S   A   L

      1467        1476        1485        1494        1503        1512
CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q   A   I

      1521        1530        1539        1548
AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'
--- --- --- --- --- --- --- --- --- --- --- ---
 S   D   H   Y   P   V   E   V   M   L   K   *
```


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FIGURE 14

(A) **pAS102**

```
LOCUS       PAS102.DNA   1566 bp    mRNA            PRI       06-MAR-1995
DEFINITION  Humanised HMFG1 Fab'2 fused to human DNase I (pAS102)
ACCESSION
NID
KEYWORDS    DNase I.
SOURCE      DNase I sequence is from assembled oligos (thus modified c/f
MHDNASE1.dna) (see Figure 2)
  ORGANISM  Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
  AUTHORS   Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.
  TITLE     Recombinant human DNase I reduces the viscosity of cystic fibrosis
            sputum
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  MEDLINE   91067672
BASE COUNT      345 a    469 c    440 g    312 t
ORIGIN

     1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
    61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
   121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
   181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
   241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
   301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
   361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
   421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
   481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
   541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
   601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
   661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
   721 GACAAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGGAGCGGC
   781 GGGCTGAAGA TCGCAGCCTT CAACATCCAG ACATTTGGGG AGACCAAGAT GTCCAATGCC
   841 ACCCTCGTCA GCTACATTGT GCAGATCCTG AGCCGCTACG ACATCGCCCT GGTCCAGGAG
   901 GTCAGAGACA GCCACCTGAC TGCCGTGGGG AAGCTGCTGG ACAACCTCAA TCAGGACGCA
   961 CCAGACACCT ATCACTACGT GGTCAGTGAG CCACTGGGAC GGAACAGCTA TAAGGAGCGC
  1021 TACCTGTTCG TGTACAGGCC TGACCAGGTG TCTGCGGTGG ACAGCTACTA CTACGATGAT
  1081 GGCTGCGAGC CCTGCGGGAA CGACACCTTC AACCGAGAGC CAGCCATTGT CAGGTTCTTC
  1141 TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC ATTGTTCCCC TGCATGCGGC CCCGGGGGAC
  1201 GCAGTAGCCG AGATCGACGC TCTCTATGAC GTCTACCTGG ATGTCCAAGA GAAATGGGGC
  1261 TTGGAGGACG TCATGTTGAT GGGCGACTTC AATGCGGGCT GCAGCTATGT GAGACCCTCC
  1321 CAGTGGTCAT CCATCCGCCT GTGGACAAGC CCCACCTTCC AGTGGCTGAT CCCCGACAGC
  1381 GCTGACACCA CAGCTACACC CACGCACTGT GCCTATGACA GGATCGTGGT TGCAGGGATG
  1441 CTGCTCCGAG GGGCCGTTGT TCCCGACTCG GCTCTTCCCT TTAACTTCCA GGCTGCCTAT
  1501 GGCCTGAGTG ACCAACTGGC CCAAGCCATC AGTGACCACT ATCCAGTGGA GGTGATGCTG
  1561 AAGTGA
//
```

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File : PAS102.DNA
Range : 1 - 1566 Mode : Normal
Codon Table : Universal

FIGURE 14 (B)

```
                9                  18                  27                  36                  45                  54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63                  72                  81                  90                  99                 108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117                 126                 135                 144                 153                 162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171                 180                 189                 198                 207                 216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225                 234                 243                 252                 261                 270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279                 288                 297                 306                 315                 324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333                 342                 351                 360                 369                 378
    GAC ACA GCC GTC TAT TAC TGT GCA ACA TCC TAC GAC TTT GCC TGG TTT CCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387                 396                 405                 414                 423                 432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441                 450                 459                 468                 477                 486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495                 504                 513                 522                 531                 540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549                 558                 567                 576                 585                 594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G
```

```
          603                 612                 621                 630                 639                 648
CTC TAC TCC CTC AGC ACC CTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

          657                 666                 675                 684                 693                 702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAC CTG GAC AAC AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

          711                 720                 729                 738                 747                 756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   E   P   K   S   C   D   K   T   H   T   C   C   V   E   C   P   P

          765                 774                 783                 792                 801                 810
TGC CCA GCA CCT GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   P   A   P   E   G   S   G   G   L   K   I   A   A   F   N   I   Q

          819                 828                 837                 846                 855                 864
ACA TTT GGG GAG ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   F   G   E   T   K   M   S   N   A   T   L   V   S   Y   I   V   Q

          873                 882                 891                 900                 909                 918
ATC CTG AGC CGC TAC GAC ATC GCC CTG CTC CAG GAG GTC AGA CAC AGC CAC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   L   S   R   Y   D   I   A   L   V   Q   E   V   R   D   S   H   L

          927                 936                 945                 954                 963                 972
ACT GCC GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   A   V   G   K   L   L   D   N   L   N   Q   D   A   P   D   T   Y

          981                 990                 999                1008                1017                1026
CAC TAC GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   Y   V   V   S   E   P   L   G   R   N   S   Y   K   E   R   Y   L

         1035                1044                1053                1062                1071                1080
TTC GTG TAC AGG CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   V   Y   R   P   D   Q   V   S   A   V   D   S   Y   Y   Y   D   D

         1089                1098                1107                1116                1125                1134
GGC TGC GAG CCC TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   C   E   P   C   G   N   D   T   F   N   R   E   P   A   I   V   R

         1143                1152                1161                1170                1179                1188
TTC TTC TCC CGG TTC ACA CAC CTC AGG GAG TTT CCC ATT GTT CCC CTG CAT GCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   F   S   R   F   T   H   V   R   E   F   A   I   V   P   L   H   A

         1197                1206                1215                1224                1233                1242
```


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```
GCC CCG GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   P   G   D   A   V   A   E   I   D   A   L   Y   D   V   Y   L   D

      1251        1260        1269        1278        1287        1296
GTC CAA GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   Q   E   K   W   G   L   E   D   V   M   L   M   G   D   F   N   A

      1305        1314        1323        1332        1341        1350
GGC TGC AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   C   S   Y   V   R   P   S   Q   W   S   S   I   R   L   W   T   S

      1359        1368        1377        1386        1395        1404
CCC ACC TTC CAG TGG CTC ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   T   F   Q   W   L   I   P   D   S   A   D   T   T   A   T   P   T

      1413        1422        1431        1440        1449        1458
CAC TGT GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTC CTC CGA GGG GCC GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   C   A   Y   D   R   I   V   V   A   G   M   L   L   R   G   A   V

      1467        1476        1485        1494        1503        1512
GTT CCC GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   P   D   S   A   L   P   F   N   F   Q   A   A   Y   G   L   S   D

      1521        1530        1539        1548        1557        1566
CAA CTG GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   L   A   Q   A   I   S   D   H   Y   P   V   E   V   M   L   K   *
```


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FIGURE 15

(A) pAS103

LOCUS PAS103.DNA 1560 bp mRNA PRI 06-MAR-1995
DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS103)
ACCESSION
NID
KEYWORDS DNase I.
SOURCE DNase I sequence is from assembled oligos (thus modified c/f MHDNASE1.dna)
ORGANISM Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.
TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
MEDLINE 91067672
BASE COUNT 344 a 468 c 436 g 312 t
ORIGIN

```
   1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
  61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
 601 TCCCTCAGCA GCGTCGTCAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AACTTCAGCC CAAATCTTGT
 721 GACAAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCGGGCTG
 781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC
 841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA
 901 GACAGCCACC TGACTGCCGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC
 961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG
1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC
1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG
1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA
1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG
1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG
1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC
1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC
1441 CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG
1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGTGA
//
```

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File : PAS103.DNA
Range : 1 - 1560 Mode : Normal
Codon Table : Universal

FIGURE 15 (B)

```
                9          18          27          36          45          54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63          72          81          90          99         108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117         126         135         144         153         162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171         180         189         198         207         216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225         234         243         252         261         270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279         288         297         306         315         324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333         342         351         360         369         378
    GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT CCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387         396         405         414         423         432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441         450         459         468         477         486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495         504         513         522         531         540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549         558         567         576         585         594
    GGC GCC CTG ACC ACC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   C   V   H   T   F   P   A   V   L   Q   S   S   C
```


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```
          603        612        621        630        639        648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

          657        666        675        684        693        702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

          711        720        729        738        747        756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   E   P   K   S   C   D   K   T   H   T   C   C   V   E   C   P   P

          765        774        783        792        801        810
TGC CCA GCA CCT GAA GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   P   A   P   E   G   G   L   K   I   A   A   F   N   I   Q   T   F

          819        828        837        846        855        864
GGG GAG ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   E   T   K   M   S   N   A   T   L   V   S   Y   I   V   Q   I   L

          873        882        891        900        909        918
AGC CGC TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   R   Y   D   I   A   L   V   Q   E   V   R   D   S   H   L   T   A

          927        936        945        954        963        972
GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   G   K   L   L   D   N   L   N   Q   D   A   P   D   T   Y   H   Y

          981        990        999       1008       1017       1026
GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   V   S   E   P   L   G   R   N   S   Y   K   E   R   Y   L   F   V

         1035       1044       1053       1062       1071       1080
TAC AGG CCT GAC CAG GTG TCT CCC CTC GAC AGC TAC TAC TAC CAT CAT CCC TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   R   P   D   Q   V   S   A   V   D   S   Y   Y   Y   D   D   G   C

         1089       1098       1107       1116       1125       1134
GAG CCC TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   P   C   G   N   D   T   F   N   R   E   P   A   I   V   R   F   F

         1143       1152       1161       1170       1179       1188
TCC CGG TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   R   F   T   E   V   R   E   F   A   I   V   P   L   H   A   A   P

         1197       1206       1215       1224       1233       1242
```

```
GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   D   A   V   A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q

      1251        1260        1269        1278        1287        1296
GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   K   W   G   L   E   D   V   M   L   M   G   D   F   N   A   G   C

      1305        1314        1323        1332        1341        1350
AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   Y   V   R   P   S   Q   W   S   S   I   R   L   W   T   S   P   T

      1359        1368        1377        1386        1395        1404
TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   Q   W   L   I   P   D   S   A   D   T   T   A   T   P   T   H   C

      1413        1422        1431        1440        1449        1458
GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CCA CGG GCC GTT GTT CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   Y   D   R   I   V   V   A   G   M   L   L   R   G   A   V   V   P

      1467        1476        1485        1494        1503        1512
GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   S   A   L   P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L

      1521        1530        1539        1548        1557
GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   Q   A   I   S   D   H   Y   P   V   E   V   M   L   K   *
```


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FIGURE 16

(h) pAS104

LOCUS PAS104.DNA 1560 bp mRNA PRI 06-MAR-1995
DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS104)
Position 924 G to A by ggg to gag
Linker GR instead of GG (position 777)
ACCESSION
NID
KEYWORDS DNase I.
SOURCE DNase I sequence is from assembled oligos (thus modified c/f MHDNASE1.dna)
ORGANISM Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
AUTHORS Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.
TITLE Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
MEDLINE 91067672
BASE COUNT 346 a 468 c 434 g 312 t
ORIGIN

```
   1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
  61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
 721 GACAAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCRGGCTG
 781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC
 841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA
 901 GACAGCCACC TGACTGCCGT GCAGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC
 961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG
1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC
1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG
1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA
1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG
1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG
1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC
1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC
1441 CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG
1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGTGA
//
```

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File : PAS104.DNA
Range : 1 - 1560 Mode : Normal
Codon Table : Universal

FIGURE 16(B)

```
                9          18          27          36          45          54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63          72          81          90          99         108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117         126         135         144         153         162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171         180         189         198         207         216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225         234         243         252         261         270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279         288         297         306         315         324
    ACA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333         342         351         360         369         378
    GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC CAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387         396         405         414         423         432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441         450         459         468         477         486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495         504         513         522         531         540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549         558         567         576         585         594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G
```

```
          603        612        621        630        639        648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

          657        666        675        684        693        702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

          711        720        729        738        747        756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   E   P   K   S   C   D   K   T   H   T   C   C   V   E   C   P   P

          765        774        783        792        801        810
TGC CCA GCA CCT GAA GGC AGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   P   A   P   E   G   R   L   K   I   A   A   F   N   I   Q   T   F

          819        828        837        846        855        864
GGG GAG ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   E   T   K   M   S   N   A   T   L   V   S   Y   I   V   Q   I   L

          873        882        891        900        909        918
AGC CGC TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   R   Y   D   I   A   L   V   Q   E   V   R   D   S   H   L   T   A

          927        936        945        954        963        972
GTG GAG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   E   K   L   L   D   N   L   N   Q   D   A   P   D   T   Y   H   Y

          981        990        999       1008       1017       1026
GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   V   S   E   P   L   G   R   N   S   Y   K   E   R   Y   L   F   V

         1035       1044       1053       1062       1071       1080
TAC AGG CCT GAC CAG GTC TCT CCC CTG GAC AGC TAC TAC TAC GAT GAT GGC TGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   R   P   D   Q   V   S   A   V   D   S   Y   Y   Y   D   D   C   C

         1089       1098       1107       1116       1125       1134
GAG CCC TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   P   C   G   N   D   T   F   N   R   E   P   A   I   V   R   F   F

         1143       1152       1161       1170       1179       1188
TCC CGG TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   R   F   T   E   V   R   E   F   A   I   V   P   L   H   A   A   P

         1197       1206       1215       1224       1233       1242
```


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```
GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   D   A   V   A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q

      1251        1260        1269        1278        1287        1296
GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   K   W   G   L   E   D   V   M   L   M   G   D   F   N   A   G   C

      1305        1314        1323        1332        1341        1350
AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   Y   V   R   P   S   Q   W   S   S   I   R   L   W   T   S   P   T

      1359        1368        1377        1386        1395        1404
TTC CAC TCG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   Q   W   L   I   P   D   S   A   D   T   T   A   T   P   T   H   C

      1413        1422        1431        1440        1449        1458
GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   Y   D   R   I   V   V   A   G   M   L   L   R   G   A   V   V   P

      1467        1476        1485        1494        1503        1512
GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   S   A   L   P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L

      1521        1530        1539        1548        1557
GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   Q   A   I   S   D   H   Y   P   V   E   V   M   L   K   *
```

FIGURE 17

(A) pAS105

```
LOCUS       PAS105.DNA   1578 bp    mRNA            PRI       06-MAR-1995
DEFINITION  Humanised HMFG1 Fab'2 fused to human DNase I with SV40 NLS(pAS105)
ACCESSION
NID
KEYWORDS    DNase I.
SOURCE      DNase I sequence is from assembled oligos (thus modified c/f
MHDNASE1.dna)
  ORGANISM  Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
  AUTHORS   Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.
  TITLE     Recombinant human DNase I reduces the viscosity of cystic fibrosis
            sputum
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  MEDLINE   91067672
BASE COUNT      353 a    473 c    442 g    310 t
ORIGIN

        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GCGGGCTGAA GATCGCAGCC
      781 TTCAACATCC AGACATTTGG GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT
      841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTCAGAGA CAGCCACCTG
      901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC
      961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG
     1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG
     1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC
     1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC
     1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG
     1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGGTC ATCCATCCGC
     1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA
     1381 CCCACGCACT GTGCCTATGA CAGGATCGTG GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT
     1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG
     1501 GCCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGGGGGG CGGACCCAAA
     1561 AAGAAGCGCA AGGTTTGA
//
```

NLS

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File : PAS105.DNA
Range : 1 - 1578 Mode : Normal
Codon Table : Universal

FIGURE 17 (B)

```
                9                  18                  27                  36                  45                  54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63                  72                  81                  90                  99                 108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117                 126                 135                 144                 153                 162
    GTG AAG GTG TCC TCC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171                 180                 189                 198                 207                 216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225                 234                 243                 252                 261                 270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279                 288                 297                 306                 315                 324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333                 342                 351                 360                 369                 378
    GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387                 396                 405                 414                 423                 432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA CCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441                 450                 459                 468                 477                 486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495                 504                 513                 522                 531                 540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549                 558                 567                 576                 585                 594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G
```


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```
                603                 612                 621                 630                 639                 648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

                657                 666                 675                 684                 693                 702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

                711                 720                 729                 738                 747                 756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   E   P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P

                765                 774                 783                 792                 801                 810
GAA GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   G   G   L   K   I   A   A   F   N   I   Q   T   F   G   E   T   K

                819                 828                 837                 846                 855                 864
ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG ACC CGC TAC GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   S   N   A   T   L   V   S   Y   I   V   Q   I   L   S   R   Y   D

                873                 882                 891                 900                 909                 918
ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   A   L   V   Q   E   V   R   D   S   H   L   T   A   V   G   K   L

                927                 936                 945                 954                 963                 972
CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   D   N   L   N   Q   D   A   P   D   T   Y   H   Y   V   V   S   E

                981                 990                 999                1008                1017                1026
CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   L   G   R   N   S   Y   K   E   R   Y   L   F   V   Y   R   P   D

               1035                1044                1053                1062                1071                1080
CAC CTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   V   S   A   V   D   S   Y   Y   Y   D   D   G   C   E   P   C   G

               1089                1098                1107                1116                1125                1134
AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGC TTC ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   D   T   F   N   R   E   P   A   I   V   R   F   F   S   R   F   T

               1143                1152                1161                1170                1179                1188
GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   V   R   E   F   A   I   V   P   L   H   A   A   P   G   D   A   V

               1197                1206                1215                1224                1233                1242
```


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```
GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAC AAA TCC GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q   E   K   W   G

      1251        1260        1269        1278        1287        1296
TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   E   D   V   M   L   M   G   D   F   N   A   G   C   S   Y   V   R

      1305        1314        1323        1332        1341        1350
CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   S   Q   W   S   S   I   R   L   W   T   S   P   T   F   Q   W   L

      1359        1368        1377        1386        1395        1404
ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   P   D   S   A   D   T   T   A   T   P   T   H   C   A   Y   D   R

      1413        1422        1431        1440        1449        1458
ATC GTG GTT GCA CCC ATG CTG CTC CGA CCC GCC GTT GTT CCC GAC TCG GCT CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   V   V   A   G   M   L   L   R   G   A   V   V   P   D   S   A   L

      1467        1476        1485        1494        1503        1512
CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L   A   Q   A   I

      1521        1530        1539        1548        1557        1566
AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   D   H   Y   P   V   E   V   M   L   K   G   G   G   P   K   K   K

      1575
CGC AAG GTT TGA 3'
--- --- --- ---
 R   K   V   *
```


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FIGURE 18

(A) pAS106

```
LOCUS       PAS106.DNA   1596 bp    mRNA            PRI       06-MAR-1995
DEFINITION  Humanised HMFG1 Fab'2 fused to human DNase I with SV40 NLS(pAS106)
ACCESSION
NID
KEYWORDS    DNase I.
SOURCE      DNase I sequence is from assembled oligos (thus modified c/f
MHDNASE1.dna)
  ORGANISM  Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
  AUTHORS   Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.
  TITLE     Recombinant human DNase I reduces the viscosity of cystic fibrosis
            sputum
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  MEDLINE   91067672
BASE COUNT      355 a    475 c    452 g    314 t
ORIGIN

      1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
     61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
    121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
    181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
    241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
    301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
    361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
    421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
    481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
    541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
    601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
    661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
    721 GACAAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGGAGCGGC
    781 GGGCTGAAGA TCGCAGCCTT CAACATCCAG ACATTTGGGG AGACCAAGAT GTCCAATGCC
    841 ACCCTCGTCA GCTACATTGT GCAGATCCTG AGCCGCTACG ACATCGCCCT GGTCCAGGAG
    901 GTCAGAGACA GCCACCTGAC TGCCGTGGGG AAGCTGCTGG ACAACCTCAA TCAGGACGCA
    961 CCAGACACCT ATCACTACGT GGTCAGTGAG CCACTGGGAC GGAACAGCTA TAAGGAGCGC
   1021 TACCTGTTCG TGTACAGGCC TGACCAGGTG TCTGCGGTGG ACAGCTACTA CTACGATGAT
   1081 GGCTGCGAGC CCTGCGGGAA CGACACCTTC AACCGAGAGC CAGCCATTGT CAGGTTCTTC
   1141 TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC ATTGTTCCCC TGCATGCGGC CCCGGGGGAC
   1201 GCAGTAGCCG AGATCGACGC TCTCTATGAC GTCTACCTGG ATGTCCAAGA GAAATGGGGC
   1261 TTGGAGGACG TCATGTTGAT GGGCGACTTC AATGCGGGCT GCAGCTATGT GAGACCCTCC
   1321 CAGTGGTCAT CCATCCGCCT GTGGACAAGC CCCACCTTCC AGTGGCTGAT CCCCGACAGC
   1381 GCTGACACCA CAGCTACACC CACGCACTGT GCCTATGACA GGATCGTGGT TGCAGGGATG
   1441 CTGCTCCGAG GGGCCGTTGT TCCCGACTCG GCTCTTCCCT TTAACTTCCA GGCTGCCTAT
   1501 GGCCTGAGTG ACCAACTGGC CCAAGCCATC AGTGACCACT ATCCAGTGGA GGTGATGCTG
   1561 AAGGGGGGCG GACCCAAAAA GAAGCGCAAG GTTTGA
//
```

↳ NLS

File : PAS106.DNA
Range : 1 - 1596 Mode : Normal
Codon Table : Universal

FIGURE 18 (5)

```
                9          18          27          36          45          54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63          72          81          90          99         108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117         126         135         144         153         162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171         180         189         198         207         216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225         234         243         252         261         270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279         288         297         306         315         324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333         342         351         360         369         378
    GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387         396         405         414         423         432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA CCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441         450         459         468         477         486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495         504         513         522         531         540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549         558         567         576         585         594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G
```


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```
GCC CCG CCC CAC GCA GTA CCC CAC ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   P   G   D   A   V   A   E   I   D   A   L   Y   D   V   Y   L   D

        1251        1260        1269        1278        1287        1296
GTC CAA GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   Q   E   K   W   G   L   E   D   V   M   L   M   G   D   F   N   A

        1305        1314        1323        1332        1341        1350
GGC TGC AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   C   S   Y   V   R   P   S   Q   W   S   S   I   R   L   W   T   S

        1359        1368        1377        1386        1395        1404
CCC ACC TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   T   F   Q   W   L   I   P   D   S   A   D   T   T   A   T   P   T

        1413        1422        1431        1440        1449        1458
CAC TGT GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   C   A   Y   D   R   I   V   V   A   G   M   L   L   R   G   A   V

        1467        1476        1485        1494        1503        1512
GTT CCC GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   P   D   S   A   L   P   F   N   F   Q   A   A   Y   G   L   S   D

        1521        1530        1539        1549        1557        1566
CAA CTG GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   L   A   Q   A   I   S   D   H   Y   P   V   E   V   M   L   K   G

        1575        1584        1593
GGC GGA CCC AAA AAG AAG CGC AAG GTT TGA 3'
--- --- --- --- --- --- --- --- --- ---
 G   G   P   K   K   K   R   K   V   *
```

```
          603        612        621        630        639        648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

          657        666        675        684        693        702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

          711        720        729        738        747        756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   E   P   K   S   C   D   K   T   H   T   C   C   V   E   C   P   P

          765        774        783        792        801        810
TGC CCA GCA CCT GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   P   A   P   E   G   S   G   G   L   K   I   A   A   F   N   I   Q

          819        828        837        846        855        864
ACA TTT GGG GAG ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   F   G   E   T   K   M   S   N   A   T   L   V   S   Y   I   V   Q

          873        882        891        900        909        918
ATC CTG AGC CGC TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   L   S   R   Y   D   I   A   L   V   Q   E   V   R   D   S   H   L

          927        936        945        954        963        972
ACT GCC GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   A   V   G   K   L   L   D   N   L   N   Q   D   A   P   D   T   Y

          981        990        999       1008       1017       1026
CAC TAC GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   Y   V   V   S   E   P   L   G   R   N   S   Y   K   E   R   Y   L

         1035       1044       1053       1062       1071       1080
TTC GTG TAC AGG CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   V   Y   R   P   D   Q   V   S   A   V   D   S   Y   Y   Y   D   D

         1089       1098       1107       1116       1125       1134
GGC TGC GAG CCC TGC GCC AAC CAC ACC TTC AAC CGA GAG CCA CCC ATT GTC AGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   C   E   P   C   G   N   D   T   F   N   R   E   P   A   I   V   R

         1143       1152       1161       1170       1179       1188
TTC TTC TCC CGG TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   F   S   R   F   T   E   V   R   E   F   A   I   V   P   L   H   A

         1197       1206       1215       1224       1233       1242
```


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FIGURE 19

(A) pAS107

```
LOCUS       PAS107.DNA   1590 bp    mRNA            PRI       06-MAR-1995
DEFINITION  Humanised HMFG1 Fab'2 fused to human DNase I with SV40 NLS(pAS107)
ACCESSION
NID
KEYWORDS    DNase I.
SOURCE      DNase I sequence is from assembled oligos (thus modified c/f
MHDNASE1.dna)
  ORGANISM  Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
  AUTHORS   Shak,S., Capon,D.J., Hellmiss,R., Marsters,S.A. and Baker,C.L.
  TITLE     Recombinant human DNase I reduces the viscosity of cystic fibrosis
            sputum
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  MEDLINE   91067672
BASE COUNT      354 a    474 c    448 g    314 t
ORIGIN

        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCGGGCTG
      781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC
      841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA
      901 GACAGCCACC TGACTGCCGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC
      961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG
     1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC
     1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG
     1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA
     1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG
     1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG
     1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC
     1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC
     1441 CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG
     1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGGGG
     1561 GGCGGACCCA AAAAGAAGCG CAAGGTTTGA
//
```

NLS

File : FAS107.DNA
Range : 1 - 1590 Mode : Normal
Codon Table : Universal

FIGURE 19 (B)

```
                9                  18                  27                  36                  45                  54
5'  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   G   W   S   C   I   I   L   F   L   V   A   T   A   T   G   V   H

               63                  72                  81                  90                  99                 108
    TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   Q   V   Q   L   V   Q   S   G   A   E   V   K   K   P   G   A   S

              117                 126                 135                 144                 153                 162
    GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   K   V   S   C   K   A   S   G   Y   T   F   S   A   Y   W   I   E

              171                 180                 189                 198                 207                 216
    TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   V   R   Q   A   P   G   K   G   L   E   W   V   G   E   I   L   P

              225                 234                 243                 252                 261                 270
    GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   S   N   N   S   R   Y   N   E   K   F   K   G   R   V   T   V   T

              279                 288                 297                 306                 315                 324
    AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   D   T   S   T   N   T   A   Y   M   E   L   S   S   L   R   S   E

              333                 342                 351                 360                 369                 378
    GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     D   T   A   V   Y   Y   C   A   R   S   Y   D   F   A   W   F   A   Y

              387                 396                 405                 414                 423                 432
    TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA CCC TCC ACC AAG GGC CCA TCG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   G   Q   G   T   L   V   T   V   S   S   A   S   T   K   G   P   S

              441                 450                 459                 468                 477                 486
    GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   F   P   L   A   P   S   S   K   S   T   S   G   G   T   A   A   L

              495                 504                 513                 522                 531                 540
    GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S

              549                 558                 567                 576                 585                 594
    GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G
```

```
          603                 612                 621                 630                 639                 648
CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q

          657                 666                 675                 684                 693                 702
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K

          711                 720                 729                 738                 747                 756
GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   E   P   K   S   C   D   K   T   H   T   C   C   V   E   C   P   P

          765                 774                 783                 792                 801                 810
TGC CCA GCA CCT GAA GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   P   A   P   E   G   G   L   K   I   A   A   F   N   I   Q   T   F

          819                 828                 837                 846                 855                 864
GGG GAG ACC AAG ATG TCC AAT CCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   E   T   K   M   S   N   A   T   L   V   S   Y   I   V   Q   I   L

          873                 882                 891                 900                 909                 918
AGC CGC TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   R   Y   D   I   A   L   V   Q   E   V   R   D   S   H   L   T   A

          927                 936                 945                 954                 963                 972
GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   G   K   L   L   D   N   L   N   Q   D   A   P   D   T   Y   H   Y

          981                 990                 999                1008                1017                1026
GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   V   S   E   P   L   G   R   N   S   Y   K   E   R   Y   L   F   V

         1035                1044                1053                1062                1071                1080
TAC AGG CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   R   P   D   Q   V   S   A   V   D   S   Y   Y   Y   D   D   G   C

         1089                1098                1107                1116                1125                1134
GAC CCC TCC CGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   P   C   G   N   D   T   F   N   R   E   P   A   I   V   R   F   F

         1143                1152                1161                1170                1179                1188
TCC CGG TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   R   F   T   E   V   R   E   F   A   I   V   P   L   H   A   A   P

         1197                1206                1215                1224                1233                1242
```


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```
CGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT CTC CAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   D   A   V   A   E   I   D   A   L   Y   D   V   Y   L   D   V   Q

       1251        1260        1269        1278        1287        1296
GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   K   W   G   L   E   D   V   M   L   M   G   D   F   N   A   G   C

       1305        1314        1323        1332        1341        1350
AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   Y   V   R   P   S   Q   W   S   S   I   R   L   W   T   S   P   T

       1359        1368        1377        1386        1395        1404
TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   Q   W   L   I   P   D   S   A   D   T   T   A   T   P   T   H   C

       1413        1422        1431        1440        1449        1458
GCC TAT GAC AGG ATC CTC GTT GCA GGG ATG CTC CTC CGA GGG GCC GTT GTT CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   Y   D   R   I   V   V   A   G   M   L   L   R   C   A   V   V   P

       1467        1476        1485        1494        1503        1512
GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   S   A   L   P   F   N   F   Q   A   A   Y   G   L   S   D   Q   L

       1521        1530        1539        1548        1557        1566
GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   Q   A   I   S   D   H   Y   P   V   E   V   M   L   K   G   G   G

       1575        1584
CCC AAA AAG AAG CGC AAG GTT TGA 3'
--- --- --- --- --- --- --- ---
 P   K   K   K   R   K   V   *
```


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Mammalian expression of humanised HMFG1-DNase constructs

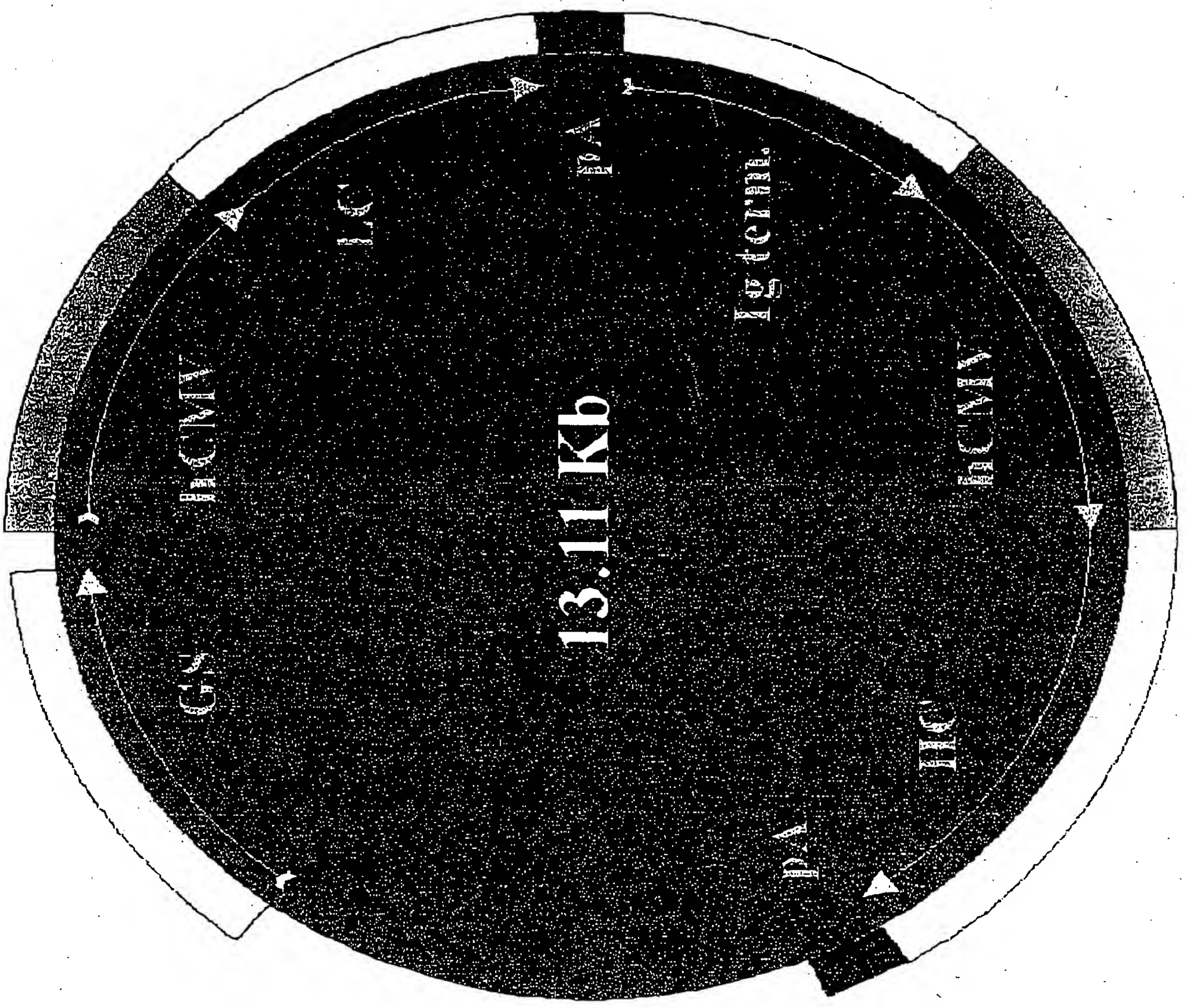
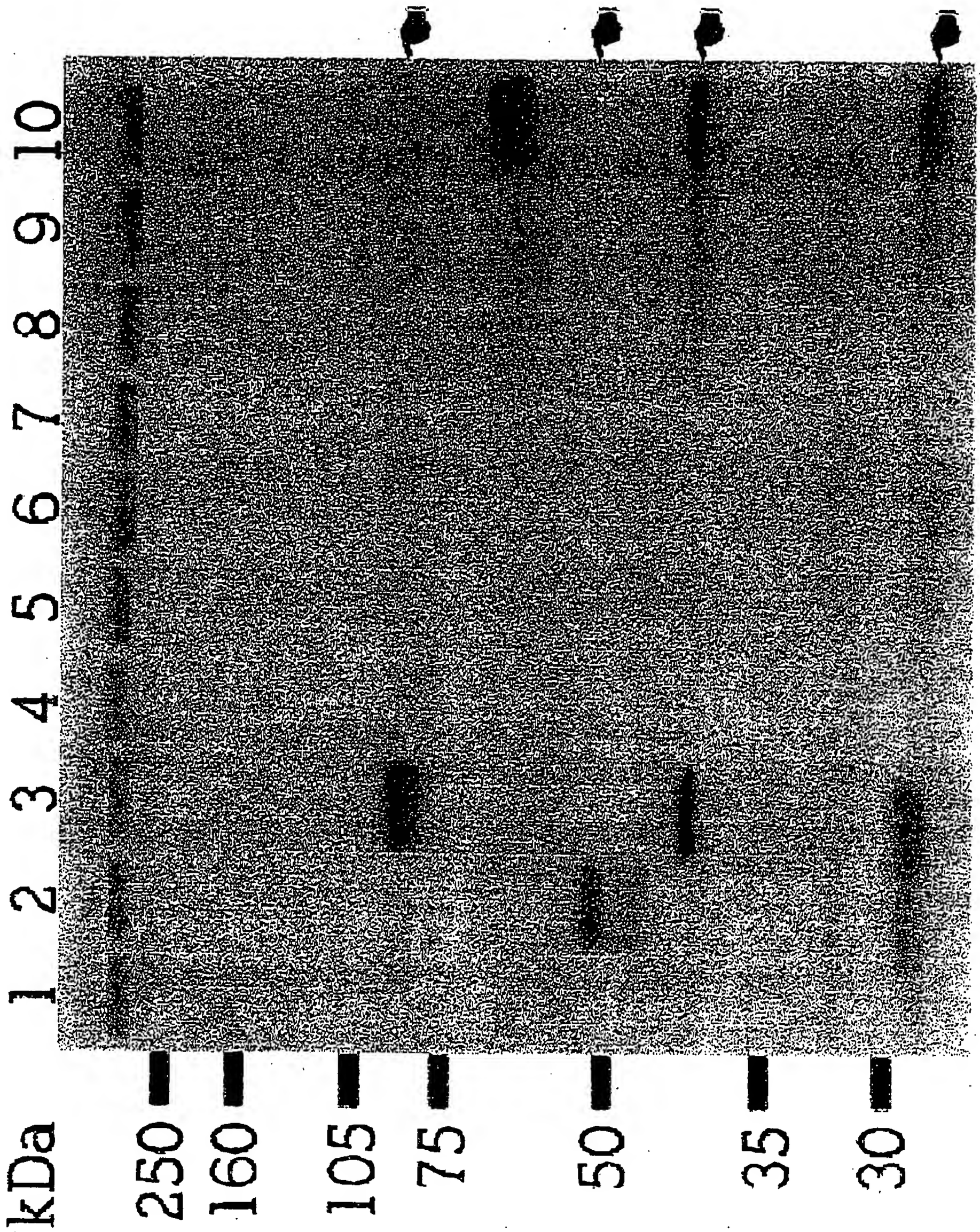


FIG 20

Fig 2(a) Immuno-precipitation of metabolically labelled transient transfectants



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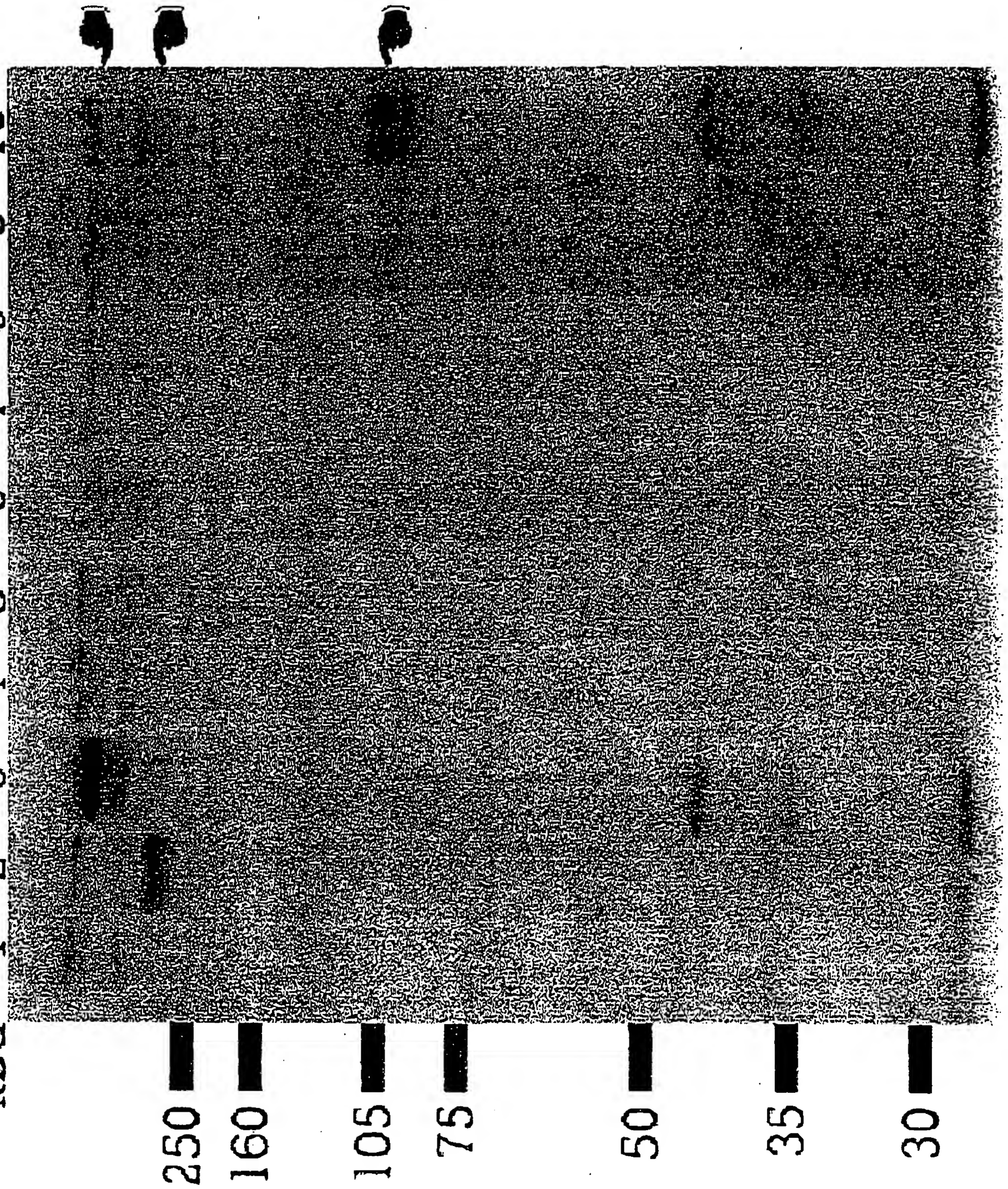
Immuno-precipitation of metabolically labelled transient transfectants
kDa 1 2 3 4 5 6 7 8 9 10
250
160
105
75
50
35
30
8% SDS-PAGE non-reducing gel

Fig 21 (b)

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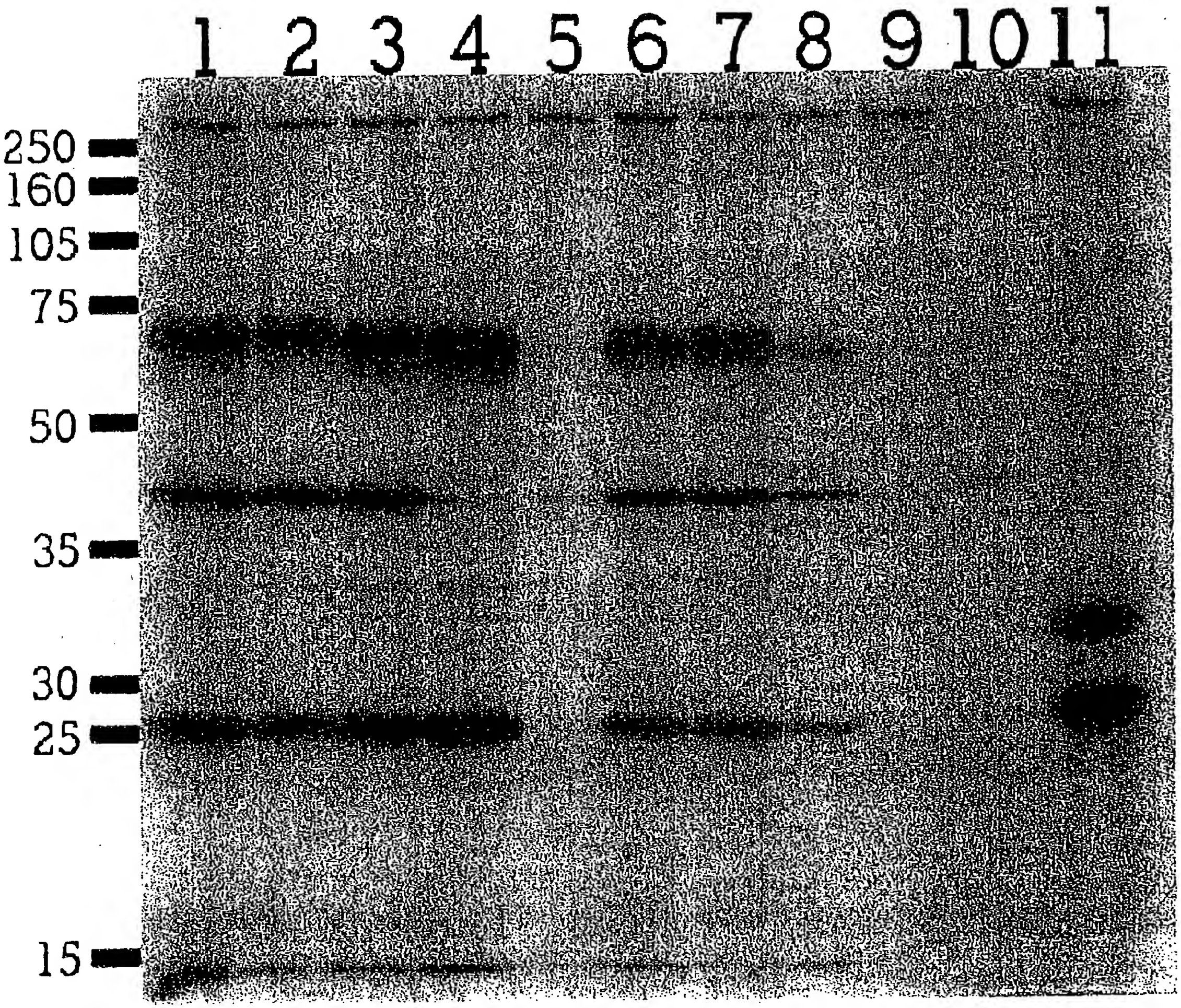
FIG 21

Immuno-precipitation of metabolically labelled transient transfectants

(C)



10% SDS-PAGE reducing gel

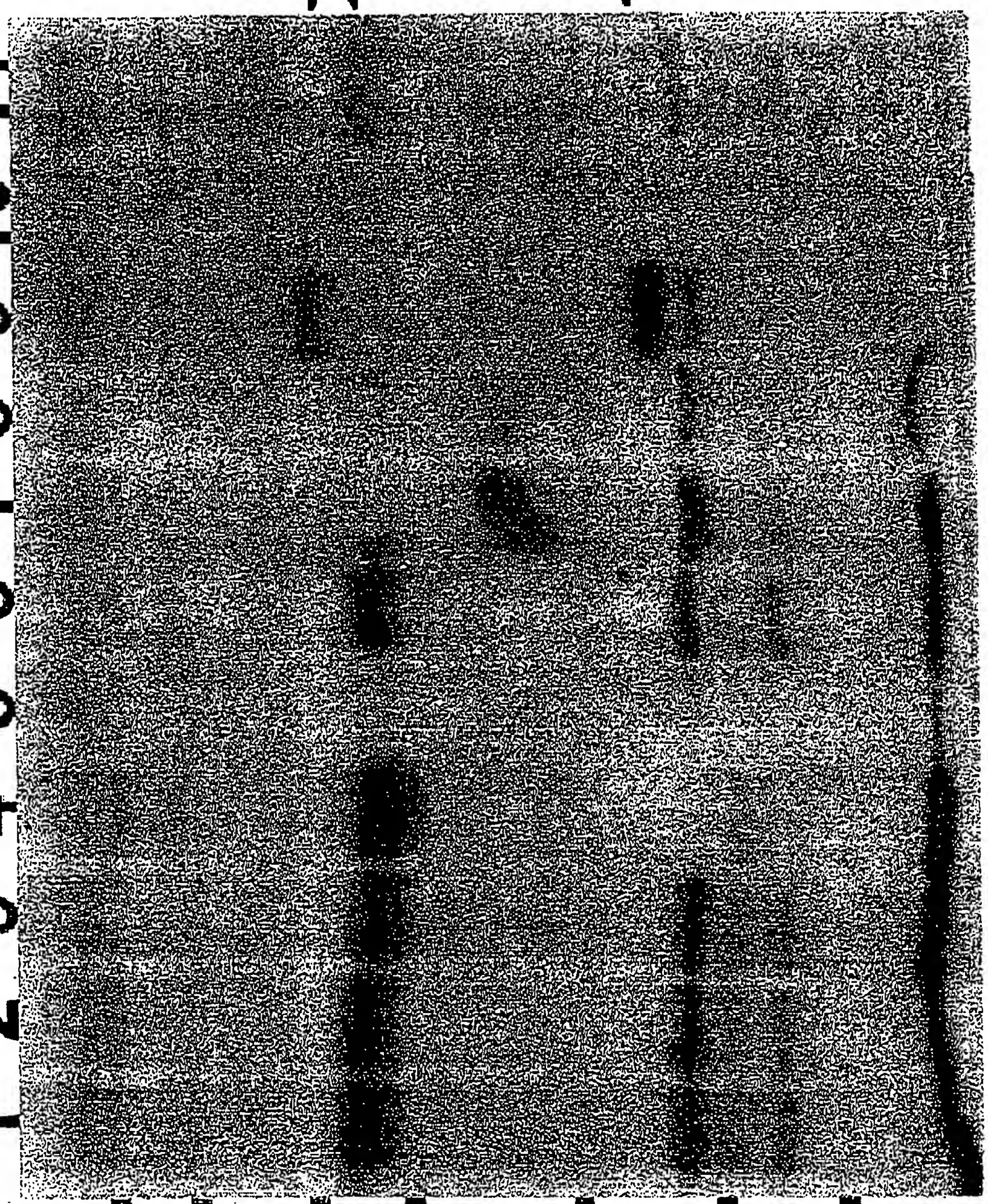

8% SDS-PAGE non-reducing gel
Immuno-precipitation of metabolically labelled transient transfectants
(D)
kDa
250
160
105
75
50
35
30
1 2
9 10 11
3 4 5 6 7 8

FIG 21

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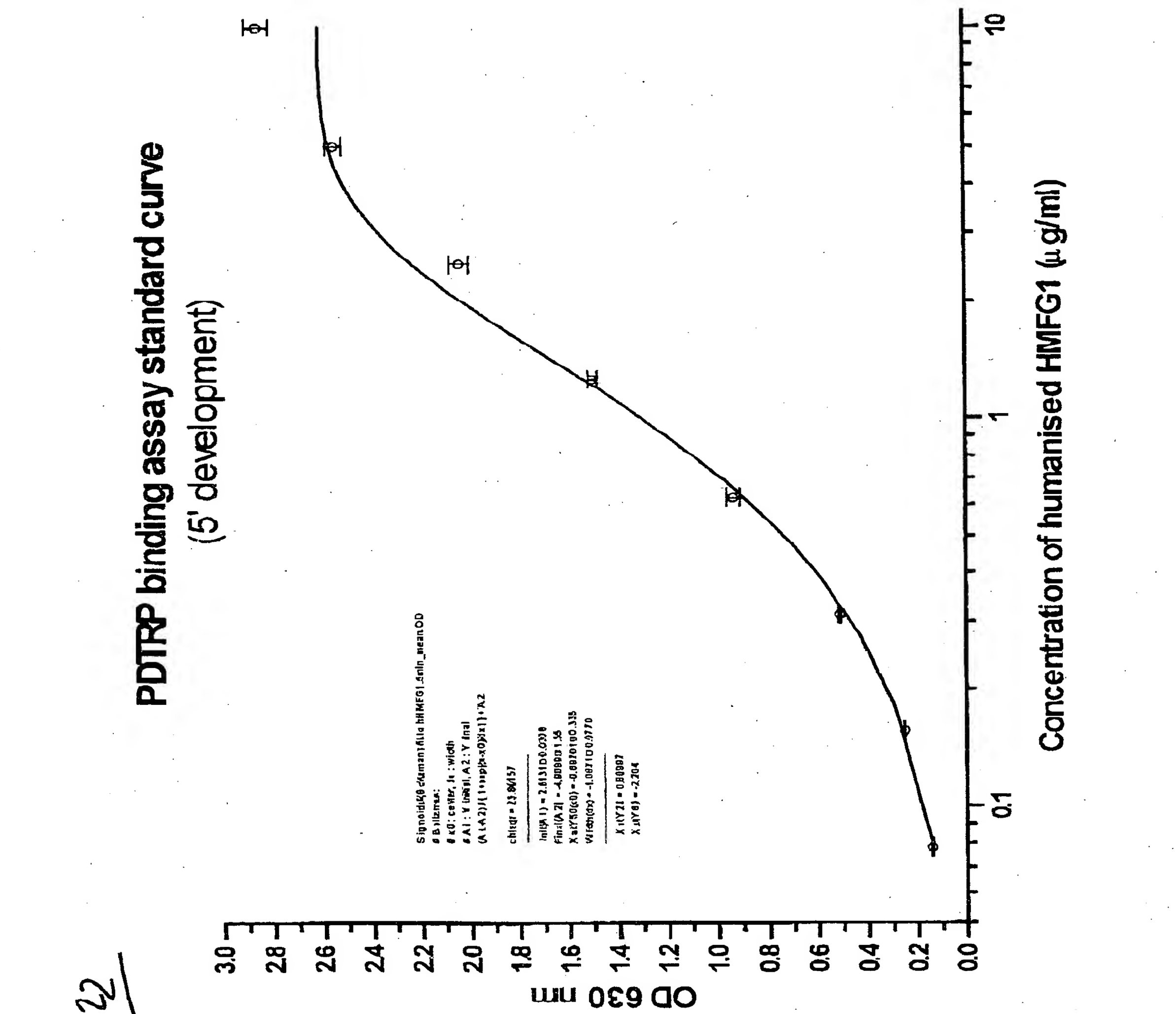
PDTRP binding assay standard curve
(5' development)
OD 630 nm
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.1
1
10
Concentration of humanised HMFG1 (µg/ml)

FIG 22

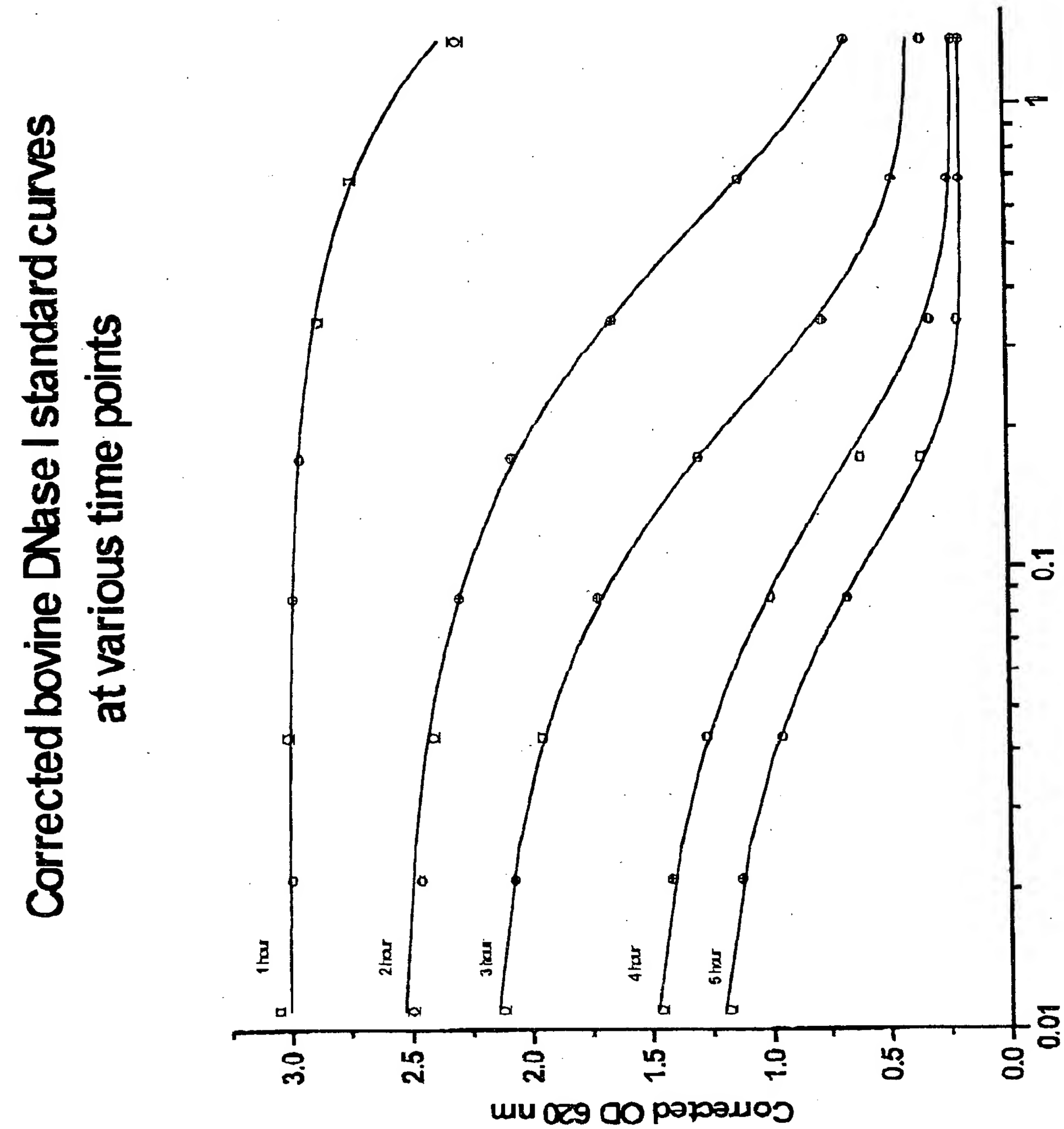
Corrected bovine DNase I standard curves at various time points
Corrected OD 620 nm
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1 hour
2 hour
3 hour
4 hour
5 hour
Bovine DNase I concentration (nM)
0.1
0.01

FIG 23

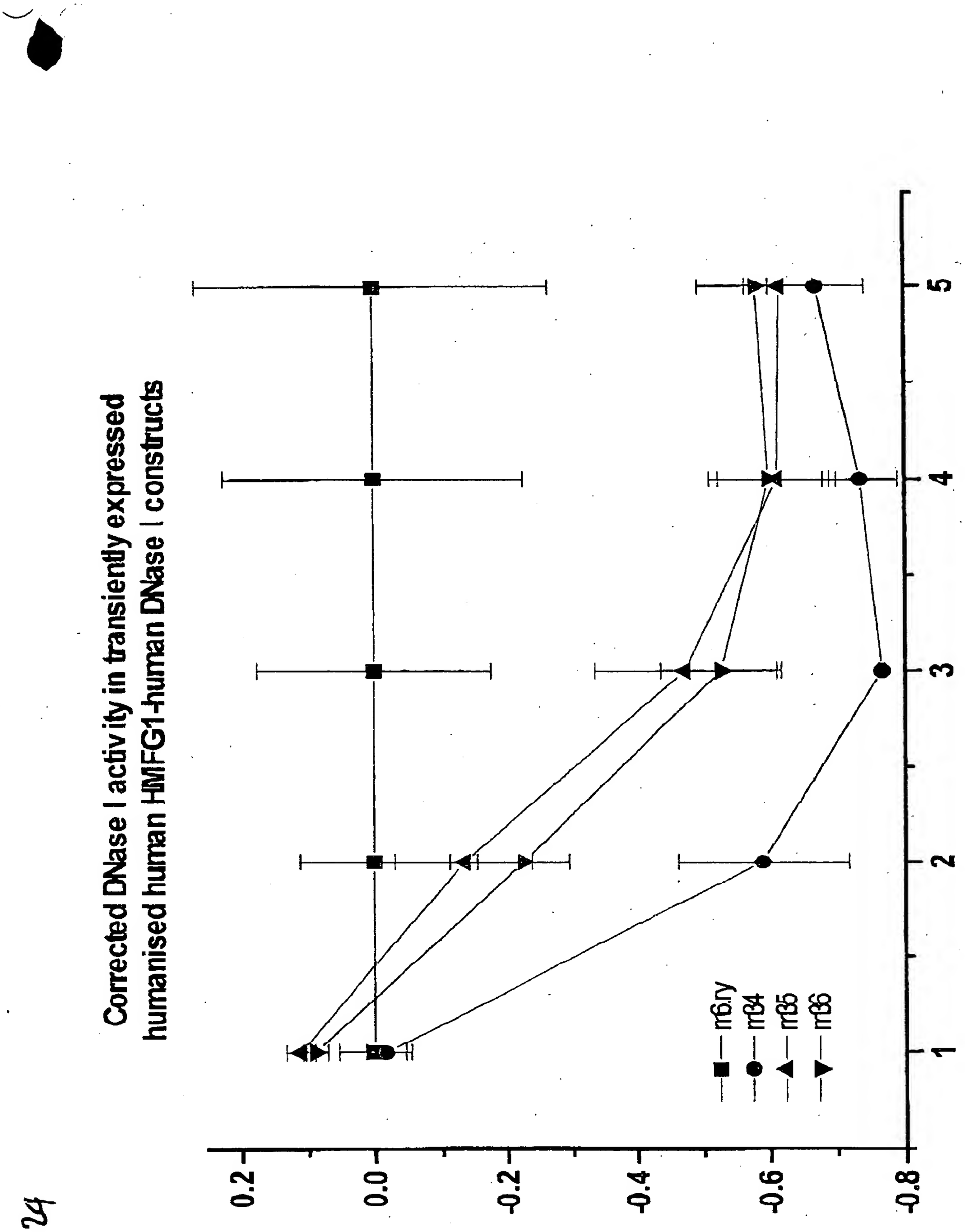
Corrected DNase I activity in transiently expressed humanised human HMFG1-human DNase I constructs
Elapsed time (h)
1
2
3
4
5
m6.ry
m34
m35
m36
Reduction in OD 620 nm
0.2
0.0
-0.2
-0.4
-0.6
-0.8

Fig 24

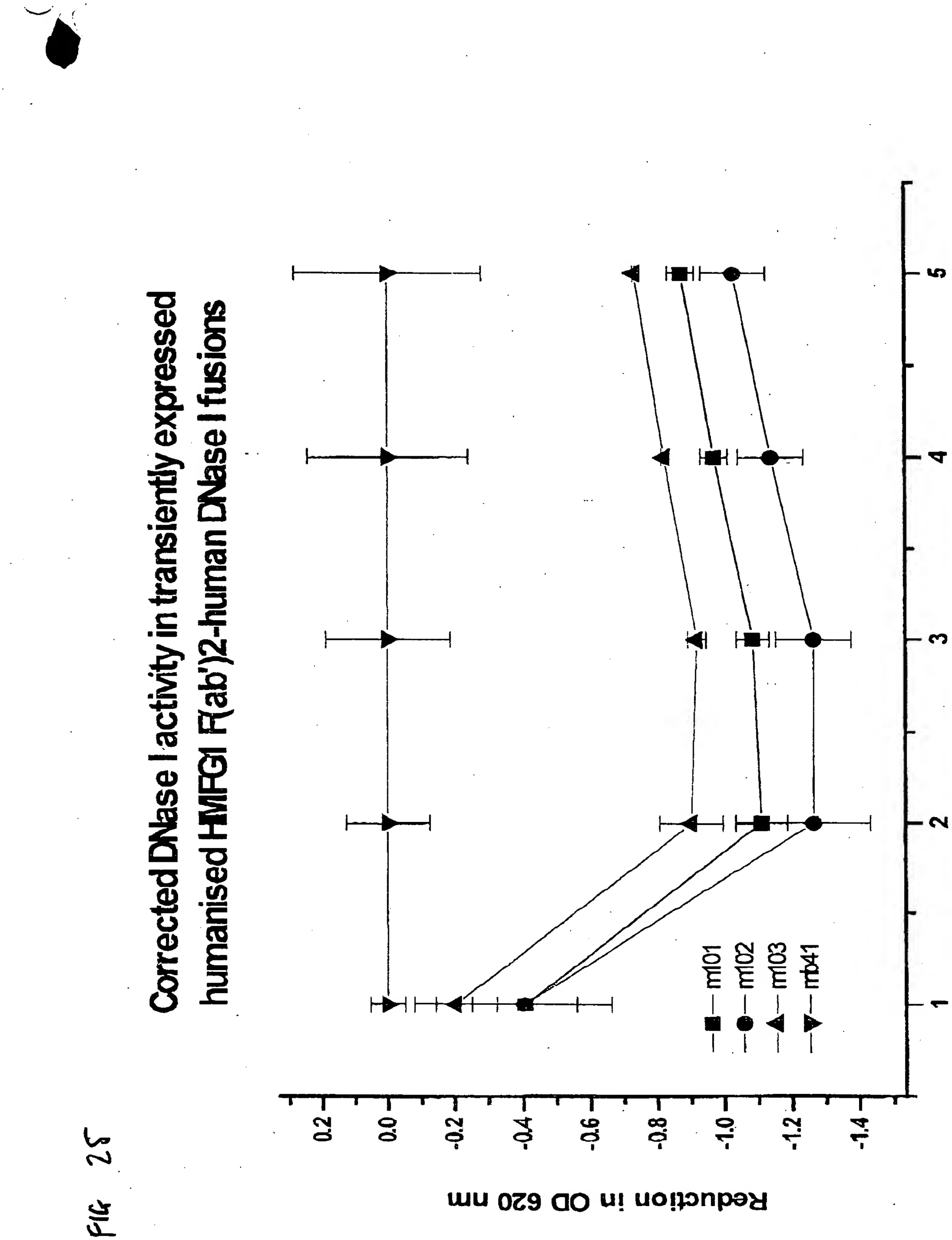
Corrected DNase I activity in transiently expressed humanised HMFG1 F(ab')2-human DNase I fusions
Reduction in OD 620 nm
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
m101
m102
m103
mb41
1
2
3
4
5
Elapsed time (h)

FIG 25

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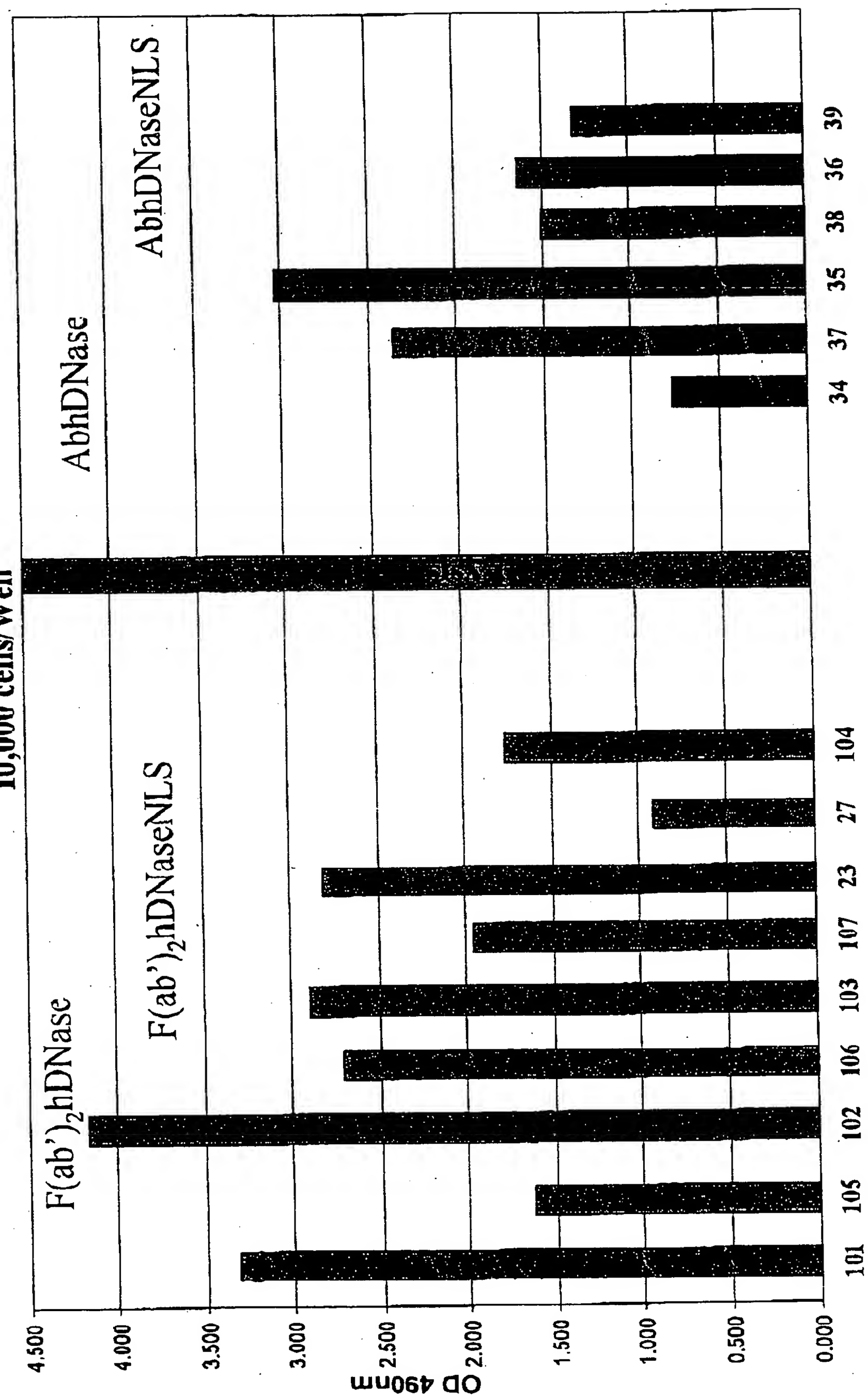
Cytotoxicity Assay
10,000 cells/Well
AbhDNase
AbhDNaseNLS
F(ab')2hDNase
F(ab')2hDNaseNLS
F(ab')2hDNase
F(ab')2hDNaseNLS
39
36
38
35
37
34
104
27
23
107
103
106
102
105
101
0.097 µg/ml of each construct
4.500
4.000
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
OD 490nm

FIG. 26

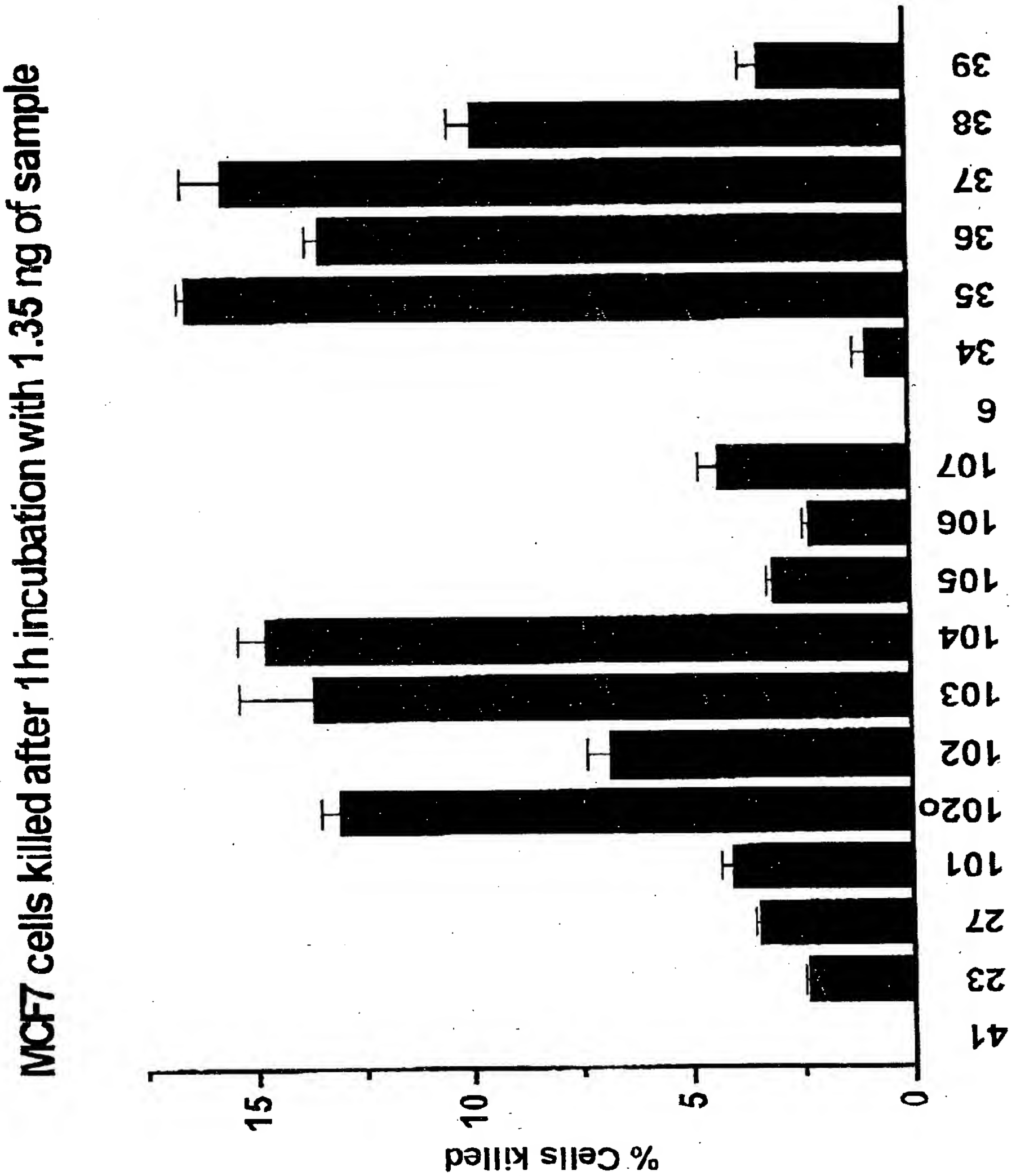
MCF7 cells killed after 1h incubation with 1.35 ng of sample
% Cells killed
0
5
10
15
Construct number
39
38
37
36
35
34
6
107
106
105
104
103
102
102o
101
27
23
41

Fig 22